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Collpas as activity hotspots for frugivorous bats (Stenodermatinae) in the Peruvian Amazon: underlying mechanisms and conservation implications

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COLLPAS AS ACTIVITY HOTSPOTS FOR FRUGIVOROUS BATS (STENODERMATINAE) IN THE PERUVIAN AMAZON: UNDERLYING MECHANISMS AND CONSERVATION IMPLICATIONS

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To my parents, Miguel and Zenaida, who have always supported me as well as encouraged me to follow my dreams and to pursue what may look impossible.

In memory of Pablo Barbadillo (1985-2008). He was a friend, a colleague, and an inspiration for many of us to fight for the conservation of our Amazonian forests.

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ABSTRACT

In western Amazonia, large numbers of frugivorous bats regularly visit natural forest clearings known locally as *collpas* (also called clay licks or mineral licks). Bats arrive at *collpas* to drink water that has accumulated in depressions created by larger mammals that consume soil. Although *collpa* visitation by bats appears relatively common in western Amazonia, little is known about its causes and its ecological implications. In this dissertation I describe general and seasonal patterns of *collpa* visitation by frugivorous bats in the Peruvian Amazon, and I investigate potential explanations for this unique behavior. Regardless of season, *collpas* seem to be activity hotspots for frugivorous bats, especially for reproductive females. Furthermore, *collpas* are visited almost exclusively by frugivorous species of the subfamily Stenodermatinae. Because some nutrients are found in low concentrations, a potential explanation for *collpa* visitation is to obtain key limited resources. *Collpas* are mineral-rich water sources. The content of selected minerals in *collpa* water, especially sodium, was significantly higher compared to other natural sources of water such as creeks, oxbow lakes, and rivers for both dry and rainy seasons. Thus, *collpas* may function as mineral sources for female reproductive frugivorous bats. Stenodermatine bats feed mostly on figs, whereas bats from the sub-family Carolliinae feed on *Piper* fruits, but also complement their diets with insects as well as other plant species. Thus, because stenodermatine species are extremely common at *collpas, collpa* visitation may be related to nutrient deficiencies in specific diets. Although there was a clear distinction in mineral and nitrogen content of *Ficus* and *Piper* fruits, they seem to provide frugivorous bats enough nitrogen (protein) and most minerals to meet their maintenance requirements. However, both fruit genera were very limited in sodium, which suggests sodium limitation for frugivorous bats in the southeastern Peruvian Amazon. Carolliine bats may be obtaining sodium from insects, whereas stenodermatine bats may use *collpas* as secondary sources of sodium, especially during reproduction. Additionally, I provide experimental evidence that demonstrates that

stenodermatine bats have a strong preference for *collpa* water. Finally, because *collpas* are important mineral sources for frugivorous bats, they should be considered important conservation targets.

CHAPTER 1. INTRODUCTION

In the Peruvian Amazon, large numbers of frugivorous bats visit *collpas* to drink water that has accumulated in soil depressions. *Collpas* are open areas in the forest where several species of geophagous mammals congregate to eat soil; in the process they make depressions from which bats drink. To date, general patterns of *collpa* visitation by non-volant mammals and birds have been described and potential explanations for geophagy have been proposed. However, little is known about the phenomenon of bat visitation, and its potential causes and consequences on bat communities.

For my dissertation, I studied bat visitation to *collpas* in the Peruvian Amazon. I developed the first detailed study that assesses general patterns of bat visitation to *collpas* across seasons, as well as potential explanations for this phenomenon.

In Chapter 1 I provide a general overview of the dissertation, followed by the main questions and hypotheses addressed by this study.

GEOPHAGY

Geophagy, the intentional consumption of soil, is a widespread behavior described for numerous vertebrates worldwide (Carbyn 1975, Emmons and Stark 1979, Terborgh 1983, Jones and Hanson 1985, Davies and Baillie 1988, Mokhtar 1990, Izawa 1993, Klaus and Schmid 1998, Klaus et al. 1998, Diamond et al. 1999, Gilardi et al. 1999, Setzl et al. 1999, Krishnamani and Mahaney 2000, Houston et al. 2001, Atwood and Weeks 2002, Holdø et al. 2002, Brightsmith and Aramburú 2004, Montenegro 2004, Mills and Milewski 2006, Ayotte et al. 2008, Brightsmith et al. 2008). Geophagous animals generally ingest soil from sites called clay licks (Gilardi et al. 1999), mineral licks (Tracy and McNaughton 1995), natural licks (Klaus and Schmid 1998, Montenegro 2004), natural mineral licks (Emmons and Stark 1979), *saladeros* (Reid et al. 2000) or salt licks (Weir 1969). In the Peruvian Amazon, native people call them by

their Quechua name: *collpas* or *ccolpas* (MacQuarrie 2001, Burger and Gochfeld 2003). For my dissertation, I will refer to the Amazonian sites as *collpas*, because this term does not imply an *a priori* function (*e.g.* sources of clay, minerals, or any other resources). Although geophagy is a widespread behavior, causes of the deliberate consumption of soil are not well understood in most cases.

Various hypotheses have been proposed to explain geophagy. For example: (1) soil may be a source of mineral supplements (Davies and Baillie 1988, Klaus and Schmid 1998). African forest elephants (*Loxodonta africana)* may obtain calcium, magnesium, manganese, phosphorus, potassium, and sodium from the consumption of soil at natural licks, where the concentrations of these minerals is higher compared to non-lick sites (Klaus and Schmid 1998). (2) Soil may provide antidiarrheal agents. Mahaney et al. (1995) suggested that soil consumed by mountain gorillas (*Gorilla gorilla beringei)* in Rwanda prevents diarrhea that would otherwise be produced by a change in their diet in the dry season. (3) Soil may provide antacid agents. Davies and Baillie (1988) suggested that the alleviation of acidosis is one of the major benefits of geophagy for red leaf monkeys (*Presbytis rubicunda*). The higher pH values of the soil consumed from termite mounds compared to the surrounding soils may make them effective antacids. (4) Soil may provide substances that absorb dietary toxins (Kreulen 1985, Diamond et al. 1999, Gilardi et al. 1999, Brightsmith et al. 2008). Gilardi et al. (1999) showed that the supplementation of clay to the diets of various *Amazona* parrots reduced the uptake of alkaloids by about 60% compared to *Amazona* parrots not fed clay supplements. In addition, Brightsmith et al. (2008) showed that clay percentages and sodium concentrations were positively correlated with parrots' preferences for specific soil patches at *collpas*. All these reasons for geophagy may be non-mutually exclusive in vertebrates.

In some cases, soil consumption by geophagous animals has also been described as highly seasonal. Temporal activity patterns of geophagous animals may be associated with the

demand for key resources at specific times of the year (Jones and Hanson 1985, Klaus and Schmidt 1998, Brightsmith 2004, Ayotte et al. 2008). In temperate forests of North America, higher mineral lick visitation occurs at the beginning of spring, when leaf flush produces a drastic change in ungulate diets, and at the end of summer, when the peak of milk production occurs (Carbyn 1975, Jones and Hanson 1985, Klaus and Schmidt 1998, Ayotte et al. 2008). In the Neotropics, Brightsmith (2004) suggested that seasonality of lick use by parrots is due to changes in diet and reproduction.

COLLPAS IN THE PERUVIAN AMAZON

In the Peruvian Amazon*, collpas* are particular open areas where the soil is exposed. They can be located on riverside cliffs or in the interior of the forest (Figure 1.1 and Figure 1.2). Their high mineral content (*e.g.*, calcium, magnesium, potassium, and sodium) compared to non-*collpa* sites (Emmons and Stark 1979, Brightsmith and Aramburú 2004, Montenegro 2004, Brightsmith et al. 2008) suggests that they may be associated with unusual geological formations. In northeastern Peru, some mineral-rich *collpas* are spatially associated with the exposure of marine sediments from the Pebas Formation (Montenegro 2004). These rich sediments are products of Middle Miocene sea incursions through the Maracaibo Basin in northern South America and the formation of the Pebas Lake in the northern Amazon Basin (Hoorn 1993, Vonhof et al. 1998). In southeastern Peru, however, no generally accepted explanation is yet available for the high mineral content of *collpa* soils. One explanation is that during the Late Miocene periodically strong tides from the Paranense Embayment from southeastern Bolivia moved salt-water to fresh-water drainages of northern Bolivia and southwestern Amazonia (Madre de Dios and Acre sub-basins) during maximal transgressions, generating estuarine tidal geological formations (Hovikoski et al. 2007). Thus, mineral-rich estuarine tidal formations may be exposed at *collpas*. An alternative explanation may be that alluvial-rich sediments are exposed at *collpas* (Linna

1993). Even so, little evidence supports the alluvial-rich sediment hypothesis. Although *collpa* formation remains partially unresolved, the use of *collpas* by geophagous animals has been reported repeatedly.

Several species of non-volant mammals and birds visit *collpas* to consume soil (Emmons and Stark 1979, Terborgh 1983, Gilardi 1996, Gilardi et al. 1999, Burger and Gochfeld 2003, Brightsmith 2004, Brightsmith and Aramburú 2004, Montenegro 2004, Tobler 2008, Tobler et al. 2009). Among mammals, five species of ungulates, two species of primates and four species of rodents have been recorded (Table 1.1, and references therein). Among birds, 19 species of parrots, four species of guans and three species of pigeons have been observed (Table 1.1, and references therein).

Some species visit *collpas* at cliffs more often than in the forest interior, and *vice versa*. Large macaws and parrots are mostly observed at *collpas* located on vertical cliffs along rivers (Burger and Gochfeld 2003, Brightsmith 2004, Brightsmith and Aramburú 2004, Brightsmith et al. 2008), whereas non-volant mammals and other birds are more common in *collpas* located in

Figure 1.1. Riverbank *collpa* on the Tambopata River in Madre de Dios, Peru (photo by Donald Brightsmith).

Figure 1.2. Forest interior *collpa* in the Los Amigos Conservation Concession in Madre de Dios, Peru.

the forest interior (Montenegro 2004, Tobler 2008, Tobler et al. 2009, Bravo pers. obs.). The vertical orientation of *collpas* on riverside cliffs may make access difficult for large mammals compared to *collpas* in the forest interior (Emmons and Stark 1979). On the other hand, the presence of a river next to *collpas* makes an open area that may facilitate the landing of large macaws and may also allow them to detect the presence of predators more easily than in a more densely shrouded forest interior *collpa* (Burger and Gochfeld 2003, Bravo pers. obs.). In addition, each species' characteristic habitat preferences may determine the selection of *collpas*. Among small parrots, only two species, rose-fronted parakeets and rock parakeets, are not observed at riverside cliff *collpas*. They frequent forest interior *collpas* usually flying under three canopy.

Large mammals that visit *collpas* in the forest interior create soil depressions where rainwater accumulates. Mammals arrive to *collpas* and consume soil from preferred areas, creating soil depressions that may become very deep (up to at least 1.7 m) over time (Bravo pers. obs.). The impact of geophagous species on *collpa* structure may depend on their body size, the frequency of their visits, and the number of individuals visiting at a time, or a combination

Table 1.1. Non-volant mammal and bird species recorded at *collpas* in the Peruvian Amazon.

Common name	Species	References
Ungulates		
Red-brocket deer	*Mazama americana*	5,6,7
Grey-brocket deer	*Mazama gouazoubira*	7
Collared peccary	*Pecari tajacu*	5,6,7
White-lipped peccary	*Tayassu pecari*	1,5,6,7
Lowland tapir	*Tapirus terrestris*	5,6,7
Primates		
Red howler monkey	*Alouatta seniculus*	2,5
Spider monkey	*Ateles belzebuth*	5
Rodents		
Brazilian porcupine	*Coendou prehensilis*	5
Paca	*Agouti paca*	5
Agouti	*Dasyprocta fuliginosa*	5
Spiny rat	*Proechimys sp.*	5
Parrots		
Mealy parrot	*Amazona farinosa*	3,4
Yellow-crowned parrot	*Amazona ochrocephala*	3,4
Blue and yellow macaw	*Ara ararauna*	4
Red and green macaw	*Ara chloropterus*	1,3,4
Scarlet macaw	*Ara macao*	1,3,4
Chestnut-fronted macaw	*Ara severus*	1,3,4
White-eyed parakeet	*Aratinga leucophthalmus*	4
Dusky-headed parakeet	*Arantinga weddellii*	3,4
Cobalt-winged parakeet	*Brotogeris cyanoptera*	1,4
Tui parakeet	*Brotogeris sanctithomae*	3,4
Dusky-billed parrotlet	*Forpus sclateri*	4
Amazonian parrotlet	*Nannopsittaca dachilleae*	4
Red-bellied macaw	*Orthopsittaca manilata*	4
White-bellied parrot	*Pionites leucogaster*	4
Blue-headed parrot	*Pionus menstruus*	3,4
Blue-headed macaw	*Primolius couloni*	4
Orange-cheeked parrot	*Pyrilia barrabandi*	3,4,5
Rose-fronted parakeet	*Pyrrhura roseifrons*	4,5
Rock parakeet	*Pyrrhura rupicola*	4
Guans		
Salvin's curassow	*Mitu salvini*	5
Spix's guans	*Penelope jacquacu*	4,5
Blue-throated piping-guan	*Pipile cumanensis*	4,5
Speckled chachalaca	*Ortalis guttata*	4
Pigeons		
Pale-vented pigeon	*Patagioenas cayennensis*	4
Plumbeous pigeon	*Patagioenas plumbea*	4
Ruddy pigeon	*Patagioenas subvinacea*	4,5

References: (1) Emmons and Stark (1979); (2) Terborgh (1983); (3) Burger and Gochfeld (2003); (4) Brightsmith (2004); (5) Montenegro (2004); (6) Tobler (2008); (7) Tobler et al. (2009).

of these variables. For example, in southeastern Peru, tapirs are the most common *collpa* visitors, followed by white-lipped peccaries and white-tailed deer (Tobler 2008). However, despite tapirs' large size and high frequency of use of *collpas*, their impact on changing the *collpa* landscape may be less dramatic than the impact of white-lipped peccaries. Single tapirs usually visit *collpas*, whereas white-lipped peccaries arrive in large herds (sometimes up to 200 individuals) trampling all ground-cover vegetation around *collpas* and creating large soil depressions (Figure 1.2). Eventually, rainwater accumulates in the depressions. Water in the *collpas* is turbid, because it gets mixed with soil by geophagous animals. Water in these depressions appears to attract some species of bats to *collpas* (Bravo pers. obs.).

Collpas are frequently visited by large numbers of frugivorous bats to drink water that has accumulated in soil depressions (Bravo et al. 2008). Dr. Louise Emmons, who has extensive experience working with Neotropical rainforest mammals, noted the phenomenon of bat visitation to both artificial and natural mineral licks in 1978, and has since observed it in many places in western Amazonia. However, to date few records have been reported. I found some anecdotal observations of this and related phenomena for Amazonian forests. For instance, Tuttle (1974) reported large numbers of stenodermatine bats drinking water form three water holes frequently visited by tapirs in the Venezuelan Amazon. In addition, he observed higher bat activity at a single small puddle on a rock where people had processed animal hides using salt and borax a few days before, compared to five other puddles. Nearer the *collpas* studied in this dissertation, in the Peruvian Amazon, Ascorra and Wilson (1991) captured large numbers of *Artibeus jamaicencis* in a "*colpa"* [sic] in northeastern Peruvian forests. In addition Ascorra et al. (1996) found clay in fecal samples of *Artibeus obscurus* in southeastern Peruvian forests, suggesting that they may have ingested water with suspended clay (or soils directly) at those *collpas*. Also, while studying *collpas* in northeastern Peru, Montenegro (2004) reported the presence of large numbers of phyllostomid bats, which activated many of the camera traps she

used to register species visiting *collpas*. In addition, two more thorough studies reported bat visitation to *collpas* in the Ecuadorian Amazon (Reid et al. 2002, Voigt et al. 2007). Although bat visitation to *collpas* has been reported since the 1970s, little is known about the causes of this behavior.

Observations of geophagy at *collpas* in the Peruvian Amazon support both mineral-based and clay-based explanations. Some studies have concluded that the presence of higher concentrations of sodium in preferred *collpa* soil compared to non-preferred soil is the main cause for geophagy (Emmons and Stark 1979, Brightsmith and Aramburú 2004, Montenegro 2004). Alternatively, Gilardi et al. (1999) concluded that the clay's ability to bind secondary metabolites is the main cause of geophagy in parrots. Because parrots feed mainly on fruits and seeds that contain high concentrations of secondary metabolites (Gilardi 1996), clay consumed at *collpas* may help to neutralize their toxic effects. Using an *in vitro* adsorption method, Gilardi et al. (1999) showed that clay could reduce up to 60% the availability of the alkaloid quinine. Furthermore, they found that clay remained in the birds' intestinal tract for more than 12 hours, suggesting cytoprotection and detoxification as the main causes of geophagy in parrots. However, Brightsmith et al. (2008) found that parrots preferred soil with higher concentrations of sodium and clay, suggesting sodium supplementation and detoxification as complementary, non-exclusive causes of geophagy in parrots. Although causes of geophagy in birds and non-volant mammals have been explored to some extent, *collpa* visitation by bats still remains as an understudied phenomenon.

BATS, *COLLPAS* AND WATER SOURCES: A PRELIMINARY ANALYSIS

Contrary to tropical rainforests, in water-limited ecosystems water often causes animal aggregations. In arid areas of temperate and tropical latitudes, several species of bats visit water pools to drink water (O'Farrel and Bradley 1970, Stoner 2001, Adams et al. 2003, Adams and

Thibault 2006, Adams and Hayes 2008). In temperate-zone summers high ambient temperatures combined with low humidity result in high rates of evaporative water loss in bats (Studier et al. 1970, Webb et al. 1995). As a consequence, bats have to replenish their water loss by drinking directly from available water pools. This is exacerbated during reproductive periods, when water demands increase (Kurta et al. 1990, Adams and Hayes 2008).

In the Peruvian Amazon, there are abundant water sources (*e.g.*, oxbow lakes, creeks, rivers) that do not appear to be highly frequented by bats, and certainly not to the extent that bats visit *collpas* (Bravo pers. obs. and Emmons, pers. comm.). Thus, it seems unlikely that bats are using *collpas* as water sources. To assess this hypothesis, I compared bat activity among four different site types: a *collpa*; a reservoir; a forest; and a gap site. I also compared chemical characteristics between *collpa* and reservoir water.

Methods

Between July-September (dry season) 2007 and February-April (wet season) 2008, I captured bats monthly using 6-m mist nets at a *collpa*, a reservoir, a forest, and a gap. A man-made dam across a stream created the reservoir that collected water for use at CICRA, the biological station run by the Amazon Conservation Association (ACA) and its Peruvian counterpart the Asociación para la Conservación de la Cuenca Amazónica (ACCA). This water reservoir was of comparable size to a *collpa* (a half-circle of 6 m diameter) and was located in an undisturbed area near the station. Thus, at the *collpa* and the reservoir I used a single 6-m mist net to capture bats, whereas in the forest and the gap I deployed 6-10 6-m mist nets. Nets were opened at dusk (~1745 h) and closed at midnight (2400 h). I identified and measured each captured bat, which after being processed was released. I calculated bat activity, defined as the number of bats captured per open net per hour, for each site in different seasons and compared them with a two-way ANOVA. Prior to the analysis, I log-transformed the activity data to meet the assumptions of the test. After the ANOVA, I used a Tukey's Honest Significant Difference method (Tukey

HSD) to compare means of bat activity among sites. In addition, from February-April 2008, I collected water monthly from the *collpa* and the reservoir to analyze the mineral content. A complete description of the methods used for water collection and analysis is found in Chapter 3. I compared the mineral concentrations of calcium, magnesium, potassium, and sodium of *collpa* and reservoir water using a one-way ANOVA with repeated measures. I log-transformed the concentrations of all minerals to meet the assumptions of the test. All analyses were made in R (Crawley 2007, R Development Core Team 2007).

Results and Discussion

Bat activity at the *collpa,* gap, and reservoir sites were higher during the wet season than during the dry season ($F_{1,16} = 7.37$, $P = 0.01$). However, in both seasons bat activity at *collpas* was significantly higher than at non-*collpa* sites ($F_{3,16} = 26.95$, $P < 0.01$). Althought bat activity was greater at the reservoir than at the forest ($P = 0.02$) and gap sites ($P = 0.01$), bat activity at the *collpa* site was significantly higher compared to other sites ($P < 0.05$ for all sites). On average, at *collpas* I captured 12 bats per net per hour, whereas at the reservoir I captured 2 bats per net per hour and at the forest and gap sites less than one individual per net per hour (Figure 1.3).

In terms of species composition, at the *collpa* all but one of the total 307 individuals captured belonged to 16 frugivorous species of the subfamily Stenodermatinae. In contrast, individuals captured in the forest, the gap and the reservoir belonged to more than one feeding guild (frugivores, insectivores, omnivores). Furthermore, among the frugivorous species captured away from the *collpa*, many individuals belonged to the subfamily Carolliinae, which was represented by only one individual at the *collpa* (Table 1.2).

Water from the *collpa* had significantly higher concentrations of all minerals (Ca: $F_{1,2} = 226.5$, $P < 0.001$; K: $F_{1,2} = 115.5$, $P < 0.001$; Mg: $F_{1,2} = 33.13$, $P < 0.001$; Na: $F_{1,2} = 453.19$,

$P < 0.001$) compared to reservoir water (Figure 1.4). Sodium concentration in *collpa* water was more than 80 times higher than the concentration at the reservoir.

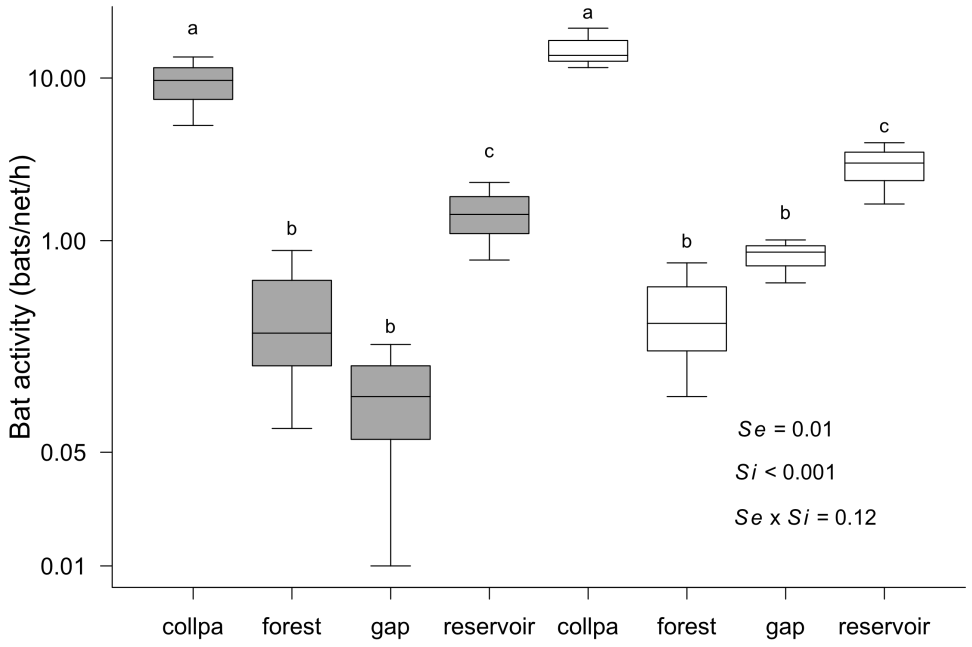

Figure 1.3. Bat activity (bats/net/h) for a *collpa*, forest, gap and reservoir site during the dry (gray boxes) and the wet (white boxes) seasons in the Peruvian Amazon. *P* values are presented for the season (*Se*) and site (*Si*) effects. Boxplots show the median, upper and lower quartiles, and highest and lowest data values. Bats were captured at each site three times during each season.

In conclusion, bat activity and species composition among sites suggests that *collpas* attract bats for a different reason than water. Large numbers of frugivorous stenodermatine bats congregate at *collpas*, whereas at the reservoir bat activity was lower and species composition was similar to the species composition in the forest and gap sites.

It is also noteworthy that frugivorous species usually do not need to ingest free water because they can obtain sufficient water from their diets. Studier and Wilson (1991) presented a "water economy budget for a 45 g *Artibeus jamaicensis*", a stenodermatine bat, where they considered zero g/day of water gains from ingested water because fruits consumed by this

Table1.2. Species richness, species abundance and total abundance of bats captured at a *collpa*, a forest, a gap, and a reservoir site in Los Amigos Conservation Concession.

Collpa		**Forest**		**Gap**		**Reservoir**	
Species	**N**	**Species**	**N**	**Species**	**N**	**Species**	**N**
Artibeus glaucus	3	*Artibeus hartii*	1	*Artibeus anderseni*	1	*Artibeus cinereus*	1
Artibeus lituratus	56	*Artibeus lituratus*	9	*Artibeus glaucus*	1	*Artibeus obscurus*	2
Artibeus obscurus	32	*Artibeus obscurus*	15	*Artibeus lituratus*	14	*Artibeus planirostris*	5
Artibeus planirostris	113	*Artibeus planirostris*	17	*Artibeus obscurus*	8	*Carollia brevicauda**	14
*Carollia perspicillata**	1	*Carollia castanea**	2	*Artibeus planirostris*	6	*Carollia perspicillata**	8
Chiroderma salvini	8	*Carollia perspicillata**	7	*Carollia brevicauda**	13	*Chiroderma salvini*	1
Chiroderma trinitatum	5	*Chiroderma trinitatum*	1	*Carollia castanea**	6	*Eptesicus brasiliensis***	5
Chiroderma villosum	10	*Chrotopterus auritus***	2	*Carollia perspicillata**	15	*Lophostoma silvicolum***	1
Platyrrhinus brachycephalus	7	*Lonchophylla thomasi***	1	*Desmodus rotundus***	1	*Miotis nigricans***	6
Platyrrhinus helleri	17	*Lophostoma silvicolum***	1	*Lonchophylla thomasi***	4	*Miotis riparius***	1
Platyrrhinus infuscus	11	*Mesophylla macconnelli*	3	*Lophostoma silvicolum***	2	*Phyllostomus hastatus***	2
Platyrrhinus sp.	3	*Micronycteris minuta***	1	*Mesophylla macconnelli*	1	*Platyrrhinus helleri*	1
Sphaeronycteris toxophyllum	5	*Phyllostomus elongatus***	6	*Phyllostomus elongatus***	5	*Platyrrhinus infuscus*	1
Uroderma bilobatum	25	*Phyllostomus hastatus***	1	*Phyllostomus hastatus***	8	*Rhinophylla pumilio**	1
Uroderma magnirostrum	5	*Platyrrhinus brachycephalus*	1	*Platyrrhinus infuscus*	2	*Sturnira lilium*	9
Vampyressa pusilla	2	*Platyrrhinus infuscus*	1	*Rhinophylla pumilio**	6		
Vampyrodes caraccioli	4	*Trachops cirrhosus***	1	*Sturnira lilium*	3		
				Sturnira tildae	1		
				*Thyroptera tricolor***	1		
				*Trachops cirrhosus***	2		
				Uroderma bilobatum	3		
				Uroderma magnirostrum	2		
TOTAL	307		70		105		58

*Species of the subfamily Carolliinae. ** Non-frugivorous species. Non-marked are species of the subfamily Stenodermatinae.

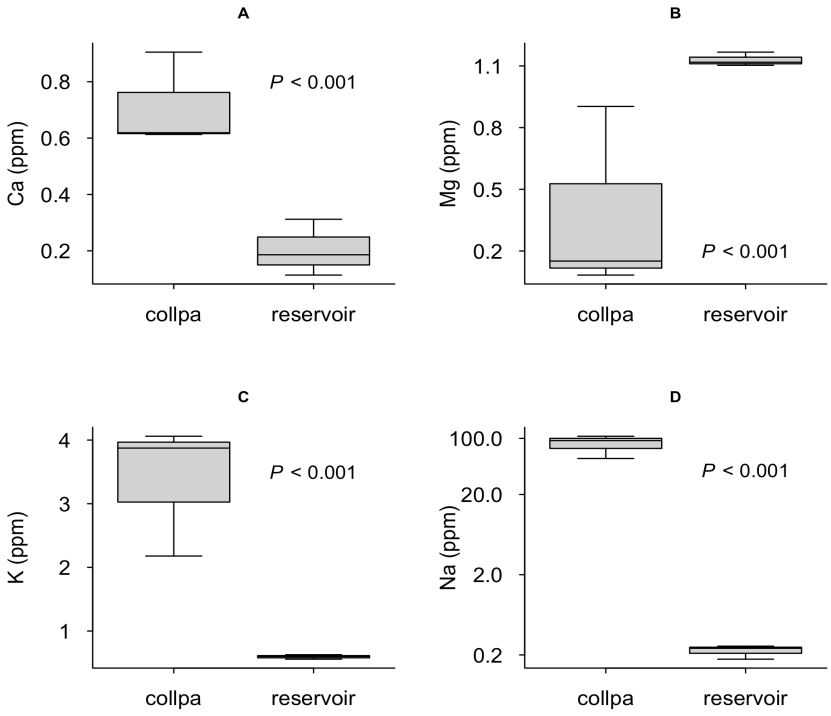

Figure 1.4. Concentrations of (A) calcium (Ca), (B) magnesium (Mg), (C) potassium (K), and (D) sodium (Na) in parts per million (ppm) for a *collpa* and an artificial reservoir at Los Amigos Conservation Concession. Boxplots show the median, upper and lower quartiles, and highest and lowest data values.

species contain 80-90% water. Thus, it is unlikely that stenodermatine frugivorous species captured at the *collpa* and the reservoir visit these places to obtain water. In a similar way, carolliines may obtain most of the water they need from *Piper* fruits that contain an average of 75% water (Fleming 1988). *Piper* was common along the stream and the reservoir; thus a potential explanation for the high number of *Carollia* species captured at the reservoir could be that they were feeding in the area. I collected fecal samples from nine individuals of *Carollia* that contained seeds of *Piper* fruits. Also, bats may have been using the stream as a flight-corridor (Jones et al. 1996). I never observed bats drinking water from the reservoir as they do at *collpas*. In addition to bat activity patterns, the differences in mineral content between the *collpa* and reservoir water suggest that it is likely that frugivorous bats may visit *collpas* to obtain minerals.

Additional research is necessary to determine potential reasons for this particular behavior, the role that *collpas* play in the ecology of bat communities in the area, and conservation priorities regarding these vertebrate communities and their resources. Because of the increased interest in bats visiting *collpas* and the small amount of available literature on this phenomenon, for my dissertation I studied the general patterns and assessed potential hypotheses for bats visiting *collpas* in the Peruvian Amazon. In addition, if *collpas* provide important resources for frugivorous bats in southeastern Peru, they should be regarded as conservation targets and may have direct implications for protecting a fully functional forest ecosystem.

STUDY SITE

General Information

My dissertation was conducted at Los Amigos Conservation Concession, located in the Department of Madre de Dios in the southeastern Peruvian Amazon (Figure 1.5). In 2001, the Peruvian government signed an agreement with the Amazon Conservation Association (ACA) and its Peruvian partner the Asociación para la Conservación de la Cuenca Amazónica (ACCA) to create the first Peruvian private conservation concession. The concession was created in an effort to protect 145,686 ha of forest in the Los Amigos River watershed, near the confluence of the Madre de Dios and Los Amigos Rivers. This concession functions as part of a natural corridor between the Manu National Park, located to the northeast of the concession, and the Tambopata National Reserve, located to the southeast (Figure 1.6). According to Holdridge et al. (1971) this region is within the Moist Humid Ecological Zone. The average annual temperature for years 2005 to 2007 ranged from 23.93 to 24.13°C, and average rainfall ranged from 2152 to 2682 mm, unevenly distributed between wet (October-April) and dry (May-September) seasons (Figure 1.7; Atrium 2008).

Figure 1.5. Los Amigos Conservation Concession located in the lowlands of the southeastern Peruvian Amazon (photo provided by the Amazon Conservation Association).

Although the concession is located in a region with many protected areas (Figure 1.6), it faces many conservation threats, as well as present and future challenges. The presence of gold in alluvial deposits of important rivers (*e.g.* Madre de Dios River) and its high price in the international and local markets have caused a massive immigration of Andean people to the lowlands of Madre de Dios. As a consequence, illegal hunting and timber extraction has also increased. In addition, in 2004 the Peruvian and Brazilian governments signed an agreement to complete the Interoceanic Highway that will connect the coast of Peru with Brazil. This highway will cross the Department of Madre de Dios. It may become a potential barrier for animal populations and may increase deforestation rates and population settlement (Delgado 2008). Therefore, biological information from the region is important to encourage local and national authorities to make decisions to protect the area.

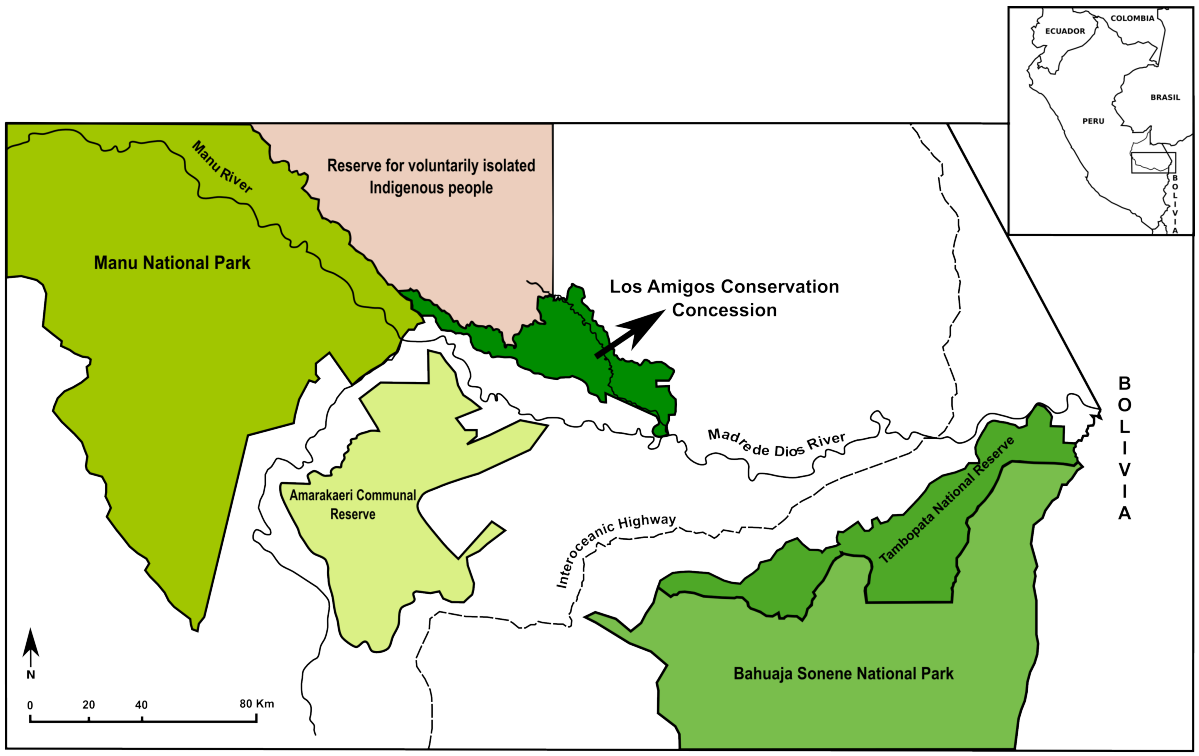

Figure 1.6. Los Amigos Conservation Concession and other protected areas in the Department of Madre de Dios in southeastern Peru (modified from a photo provided by the Amazon Conservation Association).

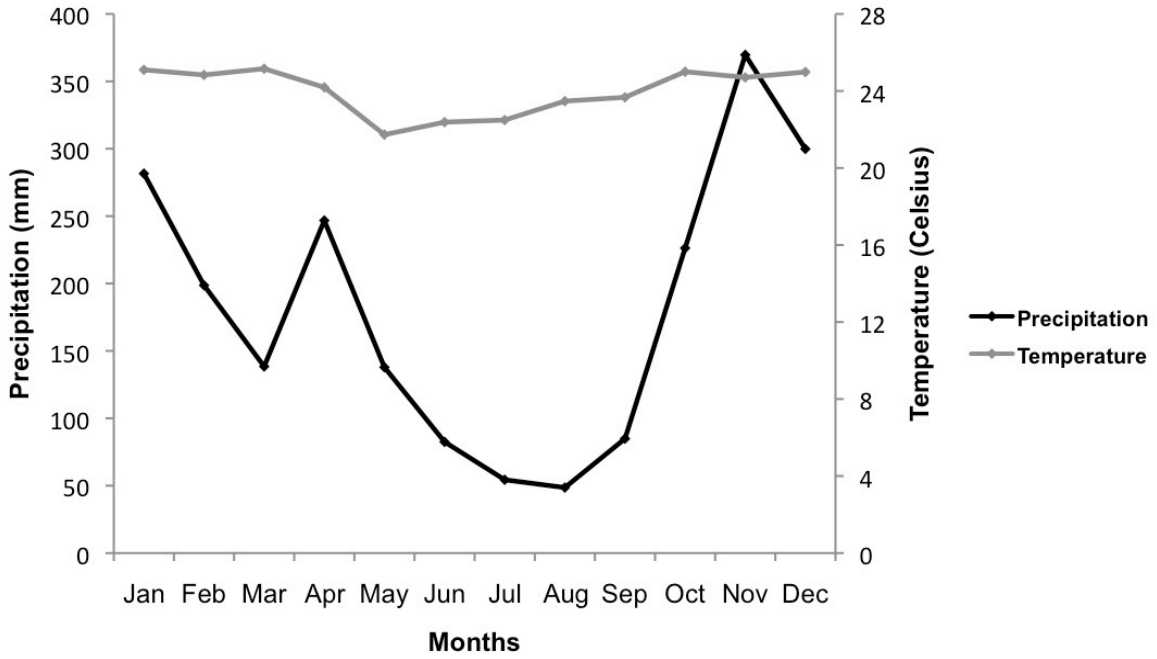

Figure 1.7. Average precipitation (C°) and temperature (mm) for the Los Amigos Biological Station in Madre de Dios, Peru from 2005-2008 (data source: ATRIUM 2008).

Geology

The Los Amigos Conservation Concession is located in the Madre de Dios sub-basin that lies on sediments deposited during the Neogene and Quaternary (~20 million years ago). Antoine et al. (2003) found different formations after developing stratigraphic columns from outcrops on the Madre de Dios and Los Amigos Rivers. The Madre de Dios River outcrop exhibited three distinctive formations: a basal layer called Ipururo Formation, deposited between Middle and Late Miocene (Räsänen 1993), a 20-m mid layer estuarine tidal sediment called the Madre de Dios Formation, that may have been deposited during Late Miocene and Early Pliocene (Antoine et al. 2003), and a top terrace. The outcrop in the Los Amigos River exhibited a 12-m layer of Madre de Dios Formation sediments and a lower top terrace compared to the Madre de Dios River. Although the origin of the geological formations in southwestern Amazonia is not completely understood, evidence suggests that the presence of tidal estuarine sediments in the area are due to sporadic tidal incursions from either or both the Pebasian or Paranense Sea transgressions in the Middle Miocene and Late Miocene to Early Pliocene, respectively (Räsänen et al. 1995, Antonie et al. 2003, Hovokoski et al. 2007). The presence of those marine sediments may explain the presence of *collpas* in the region (Emmons and Stark 1979, Terborgh 1983, Brightsmith and Aramburú 2004). In the concession alone more than 40 *collpas* have been reported along the Los Amigos River and although many species of animals visit them, very little is still known about these unique places.

Diversity at Los Amigos Conservation Concession

The concession protects a mosaic of terrestrial and aquatic habitats: *e.g.*, palm swamps; oxbow lakes; meandering rivers; white-sand forest; bamboo forest; riverine succesional forest; floodplain forest; steep-hilled and terraced terra firme forests. This extraordinary habitat diversity sustains one of the most diverse biotic communities worldwide. Pitman (2006)

compiled lists of some taxa recorded for the area: more than 500 bird species; 64 terrestrial and arboreal mammals (including 13 species of primates, five species of medium and large cats, an endangered species – the giant river otter *Pteronura brasiliensis*); 82 and ~50 species of amphibians and reptiles, respectively (von May 2004); 292 species of fish (Barthem et al. 2003, Goulding et al. 2003); 154 species of scarab beetles; 200 species of fungi (Gazis 2004, Gazis 2006); 2420 species of plants (excluding mosses and ferns) (ATRIUM 2008); 58 species of mosses (Majestyk and Janovec 2004); and 176 species of ferns. Although the species richness of bats has not been reported for the Los Amigos Conservation Concession, the number is expected to be similar to the most species-rich communities in the region.

Bat Diversity at Los Amigos Conservation Concession

Species-rich bat communities are expected in the Los Amigos Conservation Concession. Several studies conducted in the Manu National Park, contiguous to the west side of the concession, reported species-rich bat communities for the lowlands (Ascorra et al. 1991, Pacheco et al. 1993, Patterson et al. 1996, Voss and Emmons 1996). In addition, because Phyllostomidae is the most speciose family of bat species in the Neotropics, members of this family are very common in bat assemblages in southeastern Peru. For example, Voss and Emmons (1996) captured 60 bat species in Pakitza and Cocha Cashu, out of 94 expected species for the region. Twenty-one of the captured species belonged to the sub-family Stenodermatinae. Similarly, Pacheco et al. (1993) recorded 59 species, 23 stenodermatines among them, from eight sites within the lowlands of the Manu National Park.

Frugivorous bats (such as stenodermatines) play a fundamental role in maintaining functional tropical rainforest ecosystems (Fleming 1981, Fleming et al. 1981, Bizerril and Raw 1998, Hodgkison et al. 2003, Romo et al. 2004). More than 50% of plant species depend on fruit-eating animals to disperse their seeds (Howe and Smallwood 1982) and bats account for a large proportion of these frugivorous species (Fleming et al. 1987). Moreover, many tree and shrub

species have evolved specific fruit characteristics to be especially attractive to frugivorous bats. Because seed dispersal is a critical ecological process for the regeneration and maintenance of plant diversity (Harms et al. 2000, Terborgh et al. 2001), the study and conservation of bat communities is critical for the conservation of the whole forest ecosystem.

MAIN OBJECTIVE AND QUESTION

The main objective of my research is to study in detail the phenomenon of large numbers of frugivorous bats visiting *collpas* and to determine the reasons that they congregate at these places. The main question that motivates this project is: Why do frugivorous bats visit *collpas*?

SPECIFIC GOALS AND HYPOTHESES

The first main goal of my study was to determine patterns of use of *collpas* by bats in the Peruvian Amazon. I studied the general and seasonal patterns of use of *collpas* by bats and assessed how they differ from forest and gap site types. Specifically, I compared bat activity, species richness, species composition, and sex and reproductive proportions of bats between *collpas* and non-*collpa* site types.

The second main goal of my study was to determine why frugivorous bats visit *collpas* in southeastern Peru in large numbers. To reach this goal I assessed the following three hypotheses:

Hypothesis 1: *Collpas* function as sources of mineral supplementation for bats in the Peruvian Amazon.

To assess this hypothesis, I compared the chemistry of *collpa* water to comparable water bodies (*i.e.,* creeks and oxbow lakes) to determine whether the use of *collpas* is related to the presence of key nutrients such as calcium, magnesium, potassium, and sodium.

Hypothesis 2: *Collpas* provide minerals to frugivorous bats that are limited in their diets.

To assess this hypothesis, I compared the diet and mineral content of fruits consumed by bats that visit *collpas* and bats that do not visit *collpas*. Then, I compared the results to the *collpa* water mineral content to determine whether *collpas* may provide minerals limited in the bats' diet.

Hypothesis 3: Bats prefer *collpa* water, a mineral-rich water source, to mineral-poor water.

To assess this hypothesis, I experimentally tested the preference of frugivorous bats for *collpa* water compared to a mineral-poor water source.

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CHAPTER 2. *COLLPAS*: ACTIVITY HOTSPOTS FOR FRUGIVOROUS BATS (PHYLLOSTOMIDAE) IN THE PERUVIAN AMAZON[*]

INTRODUCTION

In the Neotropics, several species of vertebrates visit *collpas* to consume soil (Emmons and Stark 1979). *Collpas* (MacQuarrie 2001, alternatively *ccolpas* [Burger and Gochfeld 2003]) – also referred to as clay licks (Gilardi *et al*. 1999), mineral licks (Emmons and Stark 1979), natural licks (Klaus and Schmid 1998), and *saladeros* (Reid et al. 2002) – are open areas in the forest or on river banks where the soil is exposed. In the Peruvian Amazon, some birds (*e.g.*, cracids, parrots), monkeys (*e.g.*, black spider monkeys, red howler monkeys), ungulates (*e.g.*, collared peccaries, white-lipped peccaries, deer, tapirs), and small to medium-sized rodents (*e.g.*, spiny rats, agoutis, pacas) have been observed consuming *collpa* soils (Emmons and Stark 1979, Terborgh 1983, Gilardi et al. 1999, Burger and Gochfeld 2003; Bravo and Emmons, pers. obs.). Potential explanations for geophagy almost unanimously hypothesize that animals seek a key resource that is available in greater concentration in *collpa* soils than elsewhere (Kreulen 1985). Postulated resources include mineral elements (Emmons and Stark 1979, Brightsmith and Muñoz-Najar 2004) and dietary supplements that bind plant secondary metabolites (Gilardi et al. 1999).

In addition to birds and non-volant mammals, bats visit *collpas* in great numbers to drink water that has accumulated in depressions made by larger geophagous animals (Bravo and Emmons, pers. obs.). Despite the prominence of this behavior, I know of only one published report (Tuttle 1974) and a published abstract (Reid et al. 2002) of similar phenomena. In an intriguing paper, Tuttle (1974) reported several species of stenodermatine bats visiting two

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'water holes' in the Venezuelan Amazon that bear striking similarities to the *collpas* I have observed in Peru. These water holes were visited by tapirs with greater frequency than comparable natural pools. Tuttle (1974) reported that indigenous people who hunt tapirs in the region told him that the "noise made by the large numbers of drinking bats greatly hinders their hunting" at water holes frequented by tapirs. He also described stenodermatine bats visiting a single small pool on a rock that had been used a few days earlier for processing animal hides with borax and salts, to the exclusion of five similar pools on the same rock. *Collpa* visitation by bats was also explored in Ecuador, where the exposed soils are referred to as *saladeros* (Reid et al. 2002).

In this study, I compared patterns of use of *collpas* and non-*collpa* forest sites by bats in the Los Amigos River watershed in southeastern Peru. Specifically, I compared abundance, species richness, species composition, sex ratio, and reproductive condition of bats visiting *collpas* relative to comparable, non-*collpa* forest sites. Given the importance of frugivorous bats to seed dispersal and forest regeneration (*e.g.*, Fleming 1988), if *collpas* provide key resources for frugivorous bats, then these sites should be regarded as conservation priorities.

METHODS

Study Site

To compare patterns of use at *collpas* and non-*collpa* sites, I mist-netted bats from September through November 2005 in Los Amigos Conservation Concession, located at the confluence of Los Amigos and Madre de Dios Rivers in the Department of Madre de Dios, southeastern Peru. This private concession protects about 136,000 hectares of Amazonian forest within the Moist Humid Ecological Zone (Holdridge et al. 1971); for a more detailed overview of the region see Terborgh (1983). The average annual temperature for 2000–2006 was 21–26°C, and average

rainfall was 2700–3000 mm, unevenly distributed between the wet (Oct–Apr) and the dry (May–Sep) seasons (Centro de Investigación y Capacitación Rio Amigos, unpubl. data).

The southeastern Peruvian Amazon is a region with high bat diversity. Studies conducted in the Manu National Park, located adjacent to the west side of the Los Amigos Conservation Concession, have reported species-rich bat communities for the lowlands (Ascorra et al. 1991, Pacheco et al. 1993, Patterson et al. 1996, Voss and Emmons 1996). Community assemblages were composed mainly of species in the family Phyllostomidae, more specifically of the subfamily Stenodermatinae. For instance, Ascorra et al. (1991) reported 17 stenodermatine bats from a total of 44 species. Voss and Emmons (1996) reported 21 stenodermatine bats from a total of 60 species sampled. Based on this information, I expected a similar number of species as reported by the latter to be present in the study area.

I selected three major *collpas* located along the Los Amigos River (*Collpa* 1: 12°32′35″ S, 70°04′58″ W; *Collpa* 2: 12°30′23″ S, 70°08′55″ W; *Collpa* 3: 12°27′30″ S, 70°15′10″ W). *Collpa* size in the study area varied considerably, from < 1 m to *ca* 20 m along the longest axis. To minimize the effect of *collpa* size on bat activity among sampling sites, I chose *collpas* of similar size (*Collpa* 1: 17.4 × 8.3 m; *Collpa* 2: 18 × 7.6 m; *Collpa* 3: 16.3 × 10. 2 m) located in mature floodplain forest at *ca* 1 km from the Los Amigos river bank. To maximize our sampling area and to provide relatively independent estimates of bat activity patterns, *collpas* were spatially separated by > 8 km. At each *collpa*, the ground was mostly bare from the water edge to about 5 m, with only a few established shrubs and trees > 50 cm high. These individuals were presumably less vulnerable to trampling and browsing by large mammals than smaller plants. The absence of most vegetation is due to the activities of tapirs and large herds of peccaries that trample and/or root in the soil surrounding the *collpas*. Large mammals, such as peccaries and tapirs, were actively visiting the studied *collpas* (fresh tracks and observations). To compare bat activity at *collpas* with background activity in the forest, I established a non-*collpa* forest site in

the same mature floodplain forest where its paired *collpa* was located. Each non-*collpa* forest site had comparable vegetation structure to its paired *collpa*, 400–500 m away, but lacked standing water.

Bat Sampling

I sampled bats weekly from September to November 2005. I captured bats using 6-m mist nets at three *collpas* and paired non-*collpa* sites. To ensure that our sampling was influenced by similar variation in weather and phases of the moon, I sampled both a *collpa*/non-*collpa* pair (in random order) before moving on to the next *collpa*/non-*collpa* pair.

I used distinct protocols for sampling bats at *collpas* and non-*collpa* forest sites. At *collpas,* I set a single net *ca* 1 m from the main water pool. This net captured all the bats that two people could process effectively. In contrast, I used six to ten mist nets deployed in a zig-zag arrangement back-and-forth along and across a previously established human-made trail in the non-*collpa* forest sites. I selected relatively open sites along this trail that bats may use as flyways, and avoided cluttered areas that bats likely avoid. The use of man-made trails is a standard technique to increase capture of bats in the forest interior (Jones et al. 1996), and allowed us to use forest sites with similar vegetation structure to the *collpas* I used in this study. I generally opened the nets for 6 h at night beginning at sunset (1730 h–1745 h) until midnight. Sometimes, however, I had to close the *collpa* net before midnight because of the extreme numbers of bats captured. To minimize moon-light effects on bat activity (Morrison 1978, Lang et al. 2006), I did not set nets five nights before or after a full moon. At *collpas* and at non-*collpa* forest sites, I checked nets every 15 min, and captured bats were placed into individual cloth bags. I identified each captured bat to the level of species using diagnostic characters provided by Emmons and Feer (1997), Reid (1997), Eisenberg and Redford (1999), LaVal and Rodriguez-H (2003), Velazco (2005), as well as museum specimens examined at the Museum of Natural Science of Louisiana State University prior to embarking on the field study. I recorded sex,

reproductive status (*e.g.*, pregnant, lactating), age class (*e.g.*, juvenile, adult), weight, and forearm length of each bat captured; bats were rarely kept in cloth bags for > 30 min before processing, and never > 20 min for pregnant or lactating females. Each bat was banded with a stainless steel ball-chain necklace carrying a numbered aluminum band (Handley et al. 1991) before release.

Data Analyses

I compared bat activity – defined as the number of bats captured per net hour – at *collpas* and non-*collpa* forest sites. I calculated the mean and SE of bat activity and used a paired *t*-test in SAS to compare activity at *collpa* vs. non-*collpa* sites (Zar 1999). I tested whether the proportional representation of frugivorous bats was independent of capture site with a Chi-square Test of Independence (Sokal and Rohlf 1995). I compared bat species richness at *collpas* and non-*collpa* forest sites using rarefaction (Hurlbert 1971). I calculated the expected number of species for a given number of individuals in the program PAST (PAlaeontological STatistics, ver. 1.25, Ø. Hammer, D.A.T. Harper and P.D. Ryan, May 18, 2004) and constructed a rarefaction curve. I determined the similarity among all *collpas* and all non-*collpa* forest sites using the Bray-Curtis Index (also called the Sorensen Quantitative Index; Magurran 2004). In addition, using the same index, I determined the overall similarity between *collpas* and non-*collpa* sites. I compared species-rank abundance distributions of bats captured at *collpas* and non-*collpa* forest sites with a two-sample Kolmogorov-Smirnov test using SPSS (SPSS Inc. 1990).

In addition, I used Chi-square Tests of Independence (Sokal and Rohlf 1995) to determine whether the proportions of female vs. male bats, reproductively active vs. non-reproductive female bats, and pregnant vs. lactating reproductive female bats were independent of site of capture. I used a binomial distribution to calculate the standard deviations of the

categories analyzed, and Chi-square Goodness of Fit Tests (Sokal and Rohlf 1995) to determine whether the proportions at each site of capture differed from 50:50.

RESULTS

Bat Activity at *Collpas*

Thirty-three of a total of 60 bat species reported for this region (Voss and Emmons 1996) were captured during the sampling period (30 nights and 710 total net hours; Appendix 1). All bats captured at *collpas* and non-*collpa* forest sites belonged to the family Phyllostomidae, predominately frugivores from the subfamilies Stenodermatinae and Carolliinae. The predominance of frugivorous species at *collpas* was significantly higher than at non-*collpa* forest sites ($\chi^2 = 80.1$, $P < 0.001$). At *collpas,* 99.8 percent of the individuals were members of 24 frugivorous species and only two individuals, one of *Desmodus rotundus* and one of *Tonatia sp*., were not frugivorous (Appendix 1). In contrast, at non-*collpa* forest sites, 90 percent of the individuals were frugivorous (Appendix 1).

Bat activity at *collpas* (number of bats/net/h) was significantly greater than at non-*collpa* forest sites ($t = 16.85$, $P < 0.01$, Figure 2.1). Slightly more than 10 bats/net/h were captured at *collpas*, whereas < 1 bat/net/h was captured at non-*collpa* forest sites. Despite higher sampling intensity at non-*collpa* forest sites relative to the *collpa* sites (616 vs. 94 total open net hours), over ten times as many bats were captured at *collpas* (961 vs. 86; Appendix 1). The number of recaptured bats was very low at *collpas* as well as at non-*collpa* forest sites; only four individuals were recaptured in the former and one in the latter. This result confirms quantitatively that large numbers of bats congregate each night at *collpas*, where many bats were observed drinking the water that had accumulated in the soil depressions. It appeared that most bats arrived to a *collpa* from above the immediately adjacent canopy (as opposed to through the forest). They

maneuvered to fly close to the water pool and once they were flying above the water body, they descended and ascended back and forth to drink water.

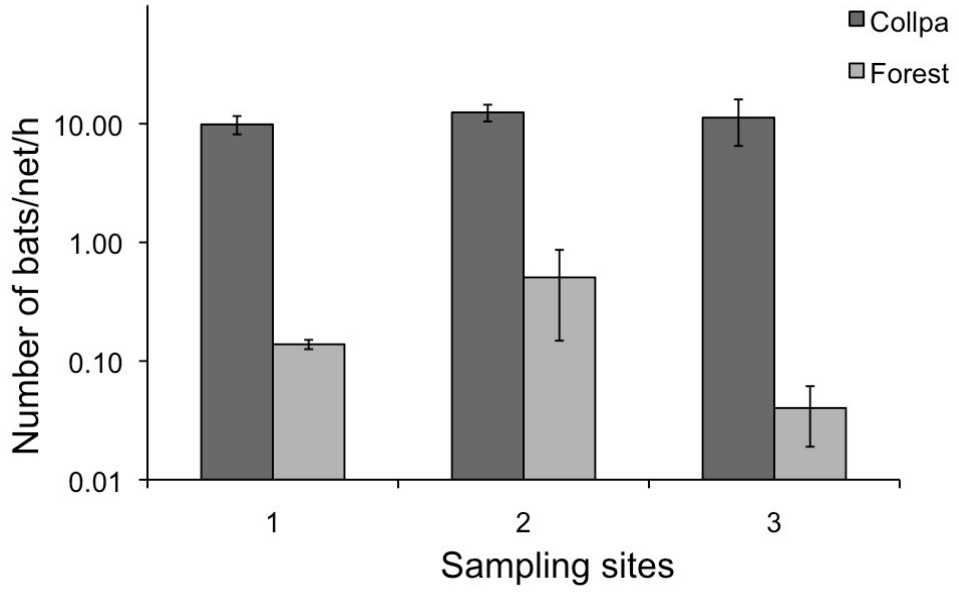


Figure 2.1. Bat captures (number of bats/net/h) ± SE for *collpas* and non-*collpa* forest sites in southeastern Peru.

Species Richness

Observed species richness was higher at *collpas* compared to non-*collpa* forest sites. Twenty-six species were captured at *collpas*, whereas only 18 species were netted at non-*collpa* forest sites (Appendix 1). The rarefaction analysis indicates that this difference in richness could be explained by the higher number of individuals captured at *collpas*. The rarefaction curves show that, for any given number of individuals, non-*collpa* forest sites have higher expected numbers of species than *collpas* (Figure 2.2). Moreover, the accumulation curve of *collpas* has an asymptotic shape, indicating that the observed number of species was close to the total number of species visiting *collpas*, whereas the curve for non-*collpa* sites is not asymptotic, showing that the total number of species expected in the area was not sampled.

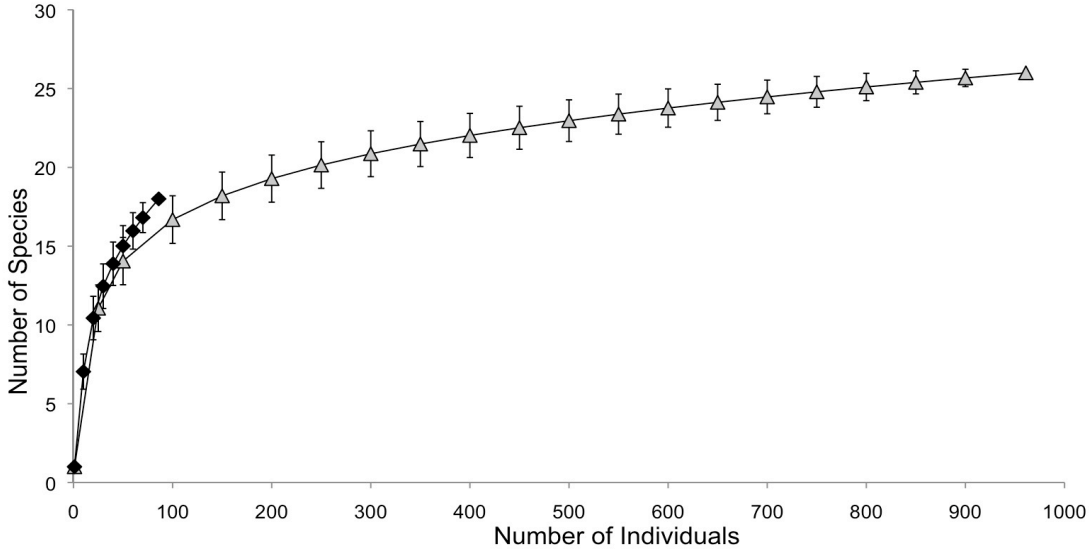


Figure 2.2. Rarefaction curves for bats visiting *collpas* and non-*collpa* forest sites in southeastern Peru. Curves represent the expected number of species for a given number of sampled individuals. Triangles represent the accumulation curve at *collpas*; diamonds represent the accumulation curve at non-*collpa* forest sites. Vertical lines represent ± SD.

Species Composition and Abundance

The pattern of species similarities was consistent across *collpas* and non-*collpa* forest sites. The three *collpas* were more similar to each other than to any non-*collpa* site, and the same pattern was seen for the non-*collpa* forest sites (Table 2.1). In contrast, low similarities were found between *collpas* and non-*collpa* forest sites (Table 2.1). When the three samples for each category were pooled, the similarity index between *collpa* and non-*collpa* sites was 0.09. A total of 33 species were captured, with 11 species common to both sites, 15 species exclusively captured at *collpas*, and 7 species captured only in non-*collpa* forest sites.

The species-rank abundance distributions of bats differed between *collpas* and non-*collpa* forest sites (Kolmogorov-Smirnov $Z = 1.52$, $P = 0.02$, Figure 2.3). At both types of sites, one very common species occurred. *Platyrrhinus helleri* represented 18 percent of the relative abundance at *collpas*, whereas *Carollia perspicillata* represented 17 percent of the relative abundance at non-*collpa* forest sites. In addition, three common species (> 10% relative

abundance) were registered at *collpas* and non-*collpa* forest sites. *Uroderma bilobatum, Artibeus lituratus,* and *A. planirostris* were relatively common in the former, whereas *Artibeus lituratus*, *Carollia brevicauda*, and *A. planirostris* were relatively common in the latter. Nevertheless, because of the higher species richness at *collpas,* more rare species occurred at these sites than at non-*collpa* forest sites. In fact, twelve of 26 species occurred at very low relative abundances at *collpas*: *Artibeus anderseni, A. cinereus, A. concolor, Carollia brevicauda, C. perspicillata, Mesophylla macconnelli, Platyrrhinus sp., Sphaeronycteris toxophyllum, Tonatia sp., Vampyressa pusilla, Vampyressa sp.,* and *Vampyrodes caraccioli.*

Table 2.1. Bray-Curtis Similarity Index among three *collpas* and three non-*collpa* forest sites. Notice in bold the low similarities between each *collpa* and each non-*collpa* forest site.

Sites	*Collpa* 1	*Collpa* 2	*Collpa* 3	Forest 1	Forest 2
Collpa 1					
Collpa 2	0.47				
Collpa 3	0.38	0.64			
Forest 1	**0.10**	**0.23**	**0.27**		
Forest 2	**0.04**	**0.08**	**0.11**	0.35	
Forest 3	**0.03**	**0.04**	**0.05**	0.33	0.38

Bats' Sex and Reproductive Condition

At *collpas*, there was a strong female sex bias compared to non-*collpa* forest sites ($\chi^2 = 32.1$, $P < 0.0001$, Figure 2.4A). More than 70 percent of bats captured at *collpas* were female ($\chi^2 = 209.4$, $P < 0.0001$), whereas about the same numbers of female and male bats were captured at non-*collpa* forest sites ($\chi^2 = 1.22$, $P = 0.26$).

More female bats were reproductively active (lactating and pregnant) than not at both *collpas* and non-*collpa* forest sites ($\chi^2 = 3.04$, $P = 0.08$, Figure 2.4B). Nevertheless, no difference was found between the proportions of lactating and pregnant females for either type of site ($\chi^2 = 0.93$, $P = 0.34$, Figure 2.4C).

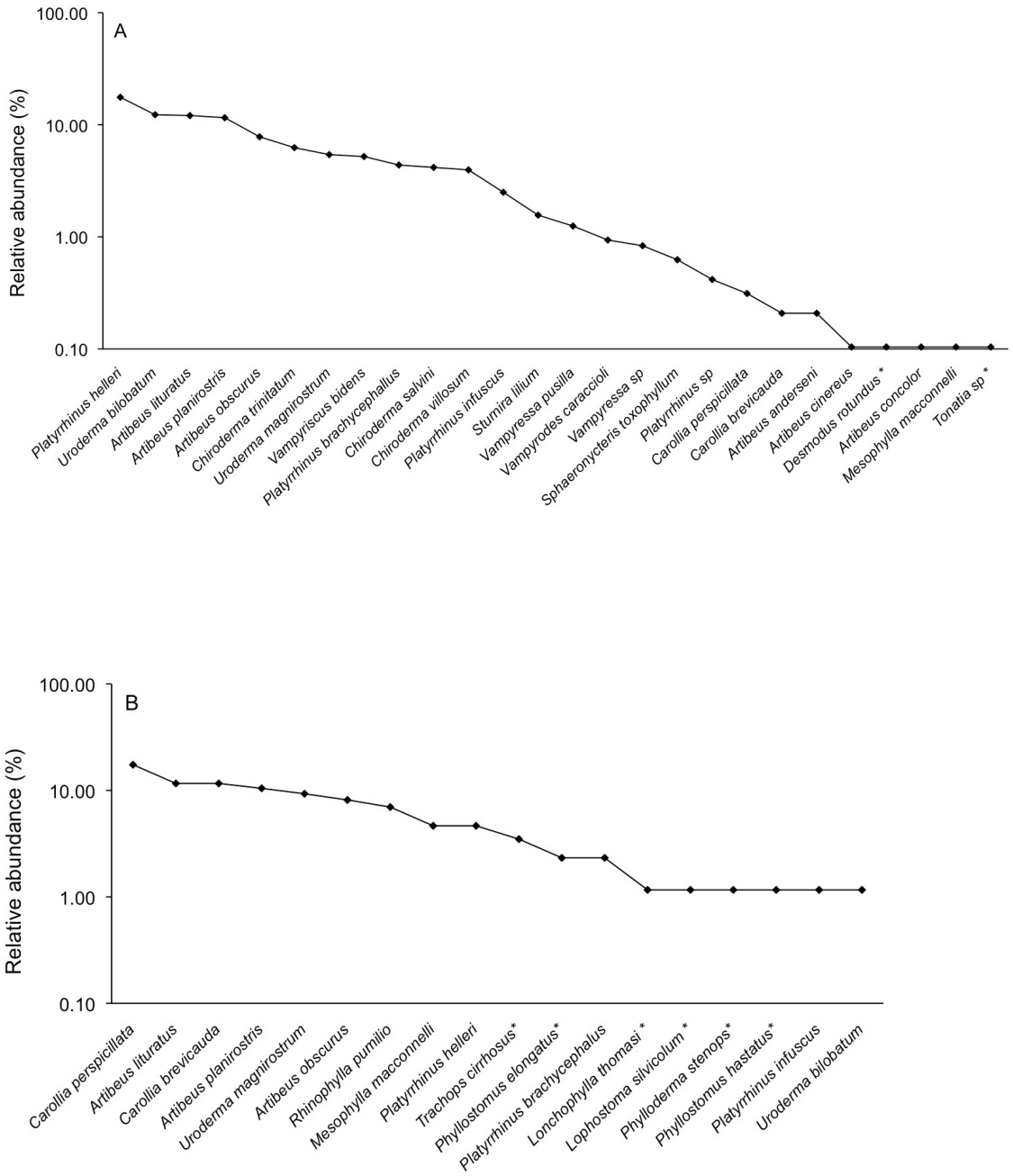


Figure 2.3. (A) Species composition and relative rank abundance of bats captured at *collpas*, and (B) bats captured at non-*collpa* forest sites. An asterisk indicates a non-frugivorous species.

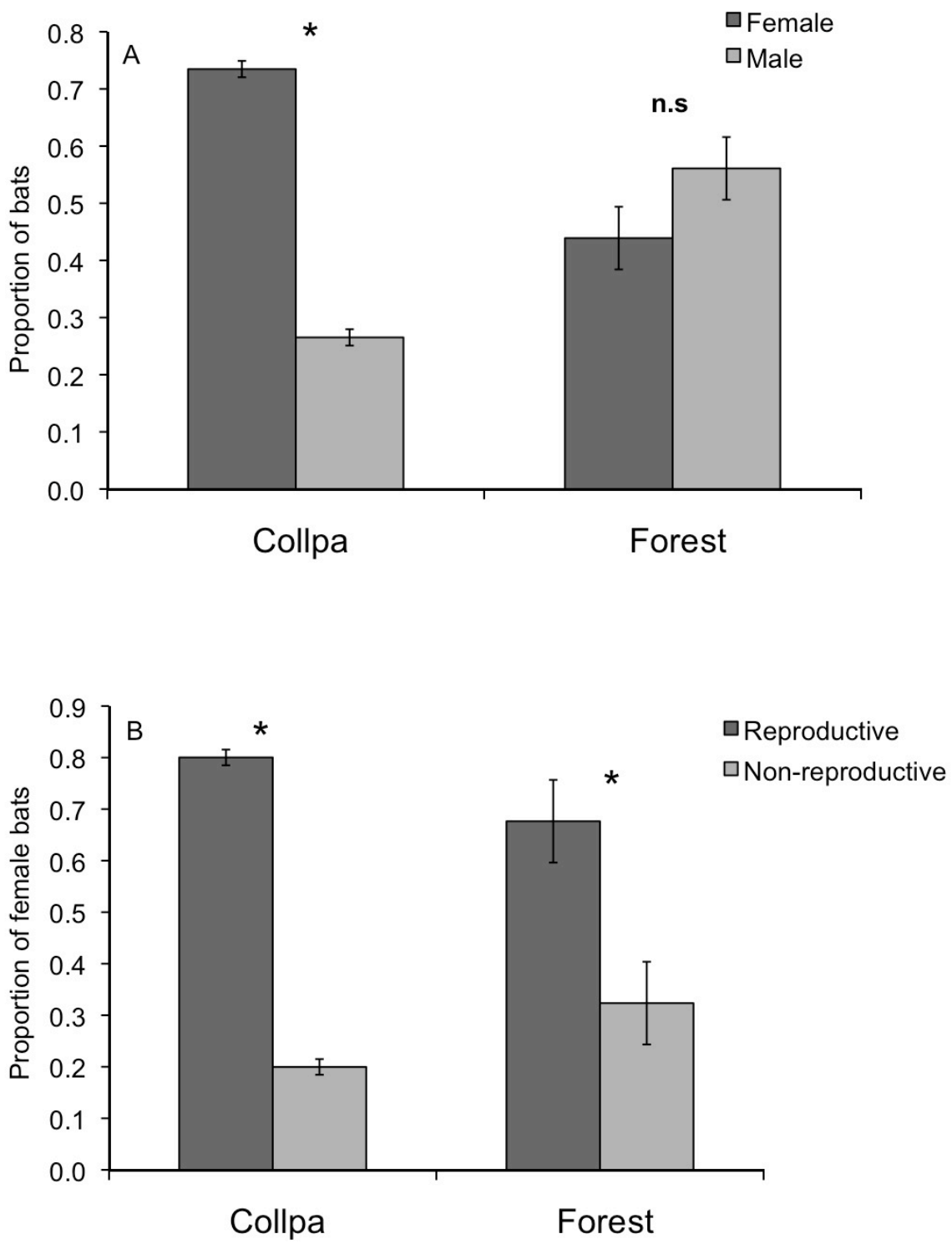
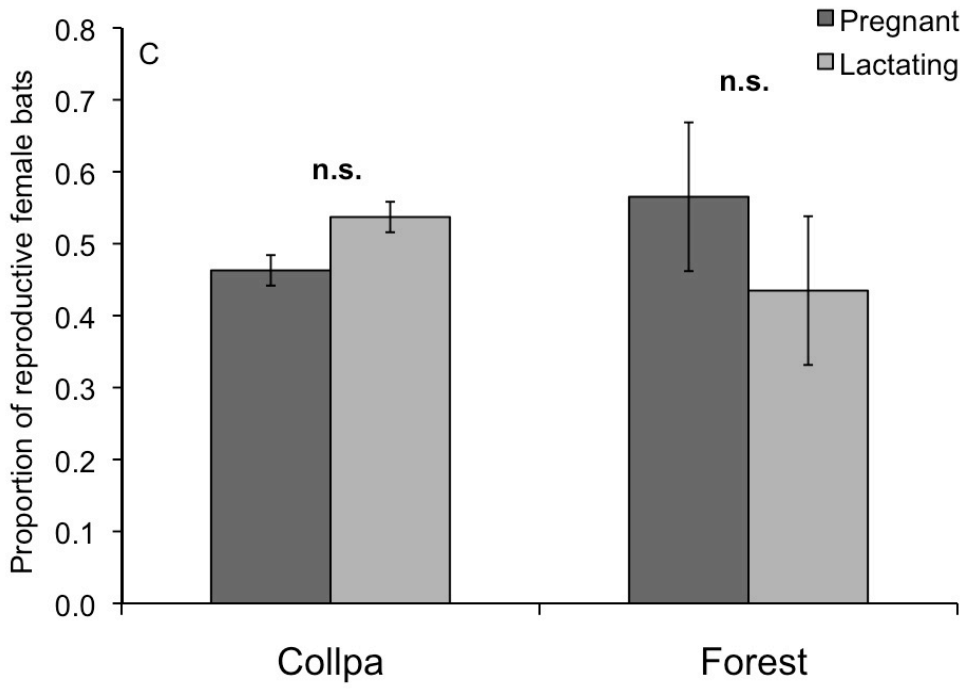


Figure 2.4. (A) Proportions of female and male bats at *collpas* and non-*collpa* forest sites; (B) Proportions of female bats in reproductive (pregnant and lactating) and non-reproductive condition at *collpas* and non-*collpa* forest sites; (C) Proportions of female bats in reproductive condition that are pregnant or lactating at *collpas* and non-*collpa* forest sites. Error bars are SD from the binomial distribution. An asterisk indicates a significant difference ($P < 0.001$).

(fig 2.4 cont'd)



DISCUSSION

Bats and *Collpas*

This study is the first to quantitatively confirm that *collpas* in the Peruvian Amazon are visited by frugivorous bats in large numbers and out of proportion to their relative abundance in local bat assemblages. Although the sampling effort at non-*collpa* forest sites was almost six and a half times higher than at *collpas*, the total number of bats captured at *collpas* was more than 10 times greater (Figure 2.1; Appendix 1). This higher bat activity at *collpas* was due to large numbers of individuals of several frugivorous species. These results suggest that bat frugivory is associated with *collpa* visitation. In addition, our results show that stenodermatine fruit bats, which are noted dietary fig-specialists (Fleming 1986, Kalko et al. 1996), seem to be most strongly associated with this behavior.

Presumably, bat species at *collpas* and non-*collpa* forest sites belong to the same local community. Although more species were captured at *collpas*, the rarefaction analysis indicates that this was only an effect of having captured more individuals there. Even so, species

abundances differed dramatically between *collpas* and non-*collpa* forest sites. There was a clear bias of frugivorous species visiting *collpas* compared to non-*collpa* forest sites. At *collpas*, only two species, *Desmodus rotundus* and *Tonatia sp.*, with one individual each, were non-frugivores. The sanguinivorous species, *D. rotundus*, may have been seeking large mammals that visit *collpas*, such as tapirs. In contrast, based on their major dietary components, six of 18 bat species were non-frugivores at non-*collpa* forest sites. Thus, bat diversity at non-*collpa* forest sites comprises species from more feeding guilds than represented at *collpas*, *e.g.*, frugivores, gleaning carnivores, gleaning insectivores, nectarivores, and omnivores. Even though several omnivores, such as *Phyllostomus hastatus*, have a strong seasonal inclusion of fruits in their diets (Gardner 1977, Giannini and Kalko 2004), their ability to eat pollen, small vertebrates, and arthropods may reduce any advantage to them of visiting *collpas*. *Collpas* might also affect composition of captured bats by drawing species that normally fly high in the forest, down to where they get captured in ground-level nets, as was suggested by Emmons et al. (2006) for pampa bat assemblages.

At non-*collpa* forest sites, species of the subfamilies Stenodermatinae and Carolliinae were the most common components of the assemblage, a general pattern for bat communities in Neotropical forests (Ascorra et al. 1996, Patterson et al. 1996, Stevens et al. 2004). In contrast, the subfamily Carolliinae was not well represented at *collpas*, where the four most abundant species belonged to the subfamily Stenodermatinae (*Platyrrhinus helleri*, *Uroderma bilobatum, Artibeus lituratus*, and *A. planirostris*). Surprisingly, species of *Carollia*, which are usually common in southeastern Peru (Ascorra et al. 1996, Patterson et al. 1996), were not among the major component species at *collpas,* yet they were at non-*collpa* forest sites.

Collpas offer a unique opportunity for the study of rare species of bats. For example, although *Sphaeronycteris toxophyllum* has a low relative abundance at *collpas,* this species is even rarer in the forest and few records have been reported (Pacheco et al. 1993, Angulo and

Diaz 2004). During the period of study, reproductive females of *S. toxophyllum* visited *collpas*. Six females were captured and five of these were pregnant. This result supports the hypothesis that *collpas* may offer important resources for reproductive female bats because even some otherwise rare species are concentrated at these particular sites.

Why Do Bats Visit *Collpas*?

Collpas in southeastern Peru clearly attract large numbers of frugivorous bat species (Phyllostomidae). Competition for limited resources generally keeps animals apart spatially or temporally, but certain limited resources can cause aggregations of animals. For instance, water draws bats to waterholes in arid landscapes (Stoner 2001, Adams and Thibault 2006), so water itself could potentially attract large numbers of bats to *collpas*, especially during the dry season. However, rivers, streams, and oxbow lakes are abundant in the lowland tropical forests of the Los Amigos watershed over the year, and frugivorous bats are not known to congregate at these bodies of water. Furthermore, frugivorous bats generally obtain nearly all their water from the fruits they eat (Fleming 1988, Studier and Wilson 1991, Wendeln et al. 2000). Thus, like other geophagous vertebrates, frugivorous bats may drink *collpa* water to obtain specific limiting nutrients, or clay to bind potential toxins.

Geophagy has been observed for many mammal and bird species worldwide (*e.g.*, Emmons and Stark 1979, Davies and Baillie 1988), but the reasons for this deliberate soil ingestion are still poorly understood. The hypotheses proposed to explain this behavior include ingestion of mineral supplements (Heymann and Hartmann 1991, Klaus and Schmid 1998), antidiarrheal agents (Mahaney et al. 1995), antacids (Davies and Ballie 1988), and substances that absorb dietary toxins (Kreulen 1985, Gilardi et al. 1999). The same hypotheses proposed to explain geophagy in mammals and birds could potentially explain why frugivorous bats drink water at the same licks. For instance, South American parrots may ingest soil to bind ingested plant secondary compounds (Gilardi et al. 1999). Alternatively, parrots may ingest soil for

nutrients (Brightsmith and Muñoz-Najar 2004), as do mammals from several vertebrate orders (*e.g.*, African savanna elephants, Asian proboscis monkeys, North American porcupines, South American white lipped peccaries, etc.) that preferentially ingest soil with high concentrations of sodium and/or calcium.

Collpa visitation by bats was strongly female biased in this study, a pattern also recorded in Ecuador by Reid et al. (2002). To fly and reproduce, bats have high nutritional requirements (Barclay 1994, Adams et al. 2003). Some species of bats seem to consume nutritionally complementary items, such as leaves, flower parts, nectar, pollen, and insects, to supplement their diets (Gardner 1977, Zortea and Lucena-Mendes 1993, Kunz and Diaz 1995). For instance, some bat species consume leaves that contain higher levels of calcium than some fruits of their diets (Ruby et al. 2000, Nelson et al. 2005). In addition, calcium, a mineral necessary to produce milk and a main component of bones, has been suggested as a limiting nutrient for female bats during reproduction (Barclay 1994, Studier and Kunz 1995, Adams et al. 2003); therefore bats may use secondary sources to obtain calcium. For example, in Colorado, large numbers of female insectivorous bats in reproductive condition visit water pools in which the concentration of calcium is high compared to non-visited pools (Adams et al. 2003). It is possible that the same phenomenon is being observed in southeastern Peru, where *collpas* may be providing mineral resources, such as calcium, for female frugivorous bats. However, whereas calcium is likely to be deficient in insectivorous diets (Bernard and Allen 1997), calcium is abundant in figs and other wild fruits (Oftedal et al. 1991, Wendeln et al. 2000), and it may be unlikely to be in short supply in frugivorous or herbivorous diets. If calcium were the nutrient sought by bats at *collpas*, then I would expect more *collpa* visitation by insectivorous, rather than by frugivorous bats (Adams et al. 2003). But calcium requirements for frugivorous bats increase significantly during reproduction and calcium provided by fig fruits may not be sufficient to cover these requirements (Barclay and Harder 2003). Thus, bats may use *collpas* as a secondary source of calcium. For

instance, a fig-specialist *Pteropus conspicillatus* in New Guinea drinks sea water, which has been postulated to serve as dietary mineral supplementation for nutrients including calcium and sodium (Iudica and Bonaccorso 2003).

Another nutrient bats may be obtaining from *collpas* is sodium, which seems to be one of the most limiting nutrients to vertebrates in the mid-continental Neotropics (Stark 1970, Emmons and Stark 1979). As a consequence, low levels of sodium in some leaves (Ruby et al. 2000) and fruits consumed by bats could generate nutritional constraints for them. Wendeln et al. (2000) suggested that frugivorous bats in Panama may specifically select sodium-rich fig fruits to make up their sodium deficits. In addition, some studies worldwide suggest that the presence of high concentrations of sodium in the soil may drive its deliberate consumption by mammals and birds (Emmons and Stark 1979, Klaus and Schmid 1998, Brightsmith and Muñoz-Najar 2004). In southeastern Peru, Emmons and Stark (1979), Gilardi et al. (1999), and Brightsmith and Muñoz-Najar (2004) found high concentrations of sodium in the clay consumed by mammals and birds. These observations leave open the possibility of bats visiting *collpas* to drink water to obtain sodium to supplement their diets.

If *collpa* visitation by bats is related to mineral supplementation and female reproduction, two reasons may explain the low abundance of Carolliinae bats observed at *collpas.* First, it may be related to the *Piper*-specialist diet of species of *Carollia* (Fleming 1986, Kalko et al. 1996, Giannini and Kalko 2004). *Piper* fruits consumed by these bats have greater energetic and nitrogen content compared to some fig fruits, which are mainly consumed by stenodermatine bats (Herbst 1986, Fleming 1988). For instance, to satisfy the basic energy and nitrogen requirement of an individual of *Carollia perspicillata*, it has to consume about 33 and 12 fruits of *Piper amalago,* respectively. In contrast, if the diet switches to *Ficus ovalis*, the bat needs to consume 77 and 82 fruits for energy and nitrogen requirements, respectively (Fleming 1988). Even the basal metabolic rate (BMR) calculated for *Piper*-specialists is much higher than the

BMR for fig-specialists (McNab 2003). However, information about nutrients besides nitrogen in *Piper* fruits, such as calcium and sodium, is not available in the literature. Thus, a *Piper*-specialized diet is not a conclusive explanation of the low number of species of *Carollia* visiting *collpas.* Second, low numbers of Carolliinae bats at *collpas* may be due to a temporal difference in reproductive season relative to other phyllostomid species captured at *collpas*. No female *Carollia* individuals were captured at *collpas*, and at non-*collpa* forest sites only two of seven female *Carollia* individuals were pregnant. Similarly, Wilson (1979) reported most of the Carollinae female bats captured in Peru in July-August as reproductively inactive. Just as we require more information on reproductive seasons and seasonal use of *collpas* by stenodermatine bats, more data are required to fully understand low rates of *collpa* visitation during the dry season by carolliine bats.

In conclusion, the large number of species and individuals of frugivorous bats visiting *collpas* suggests that *collpas* provide important resources to the community of frugivorous bats in the Peruvian Amazon, just as they do for several other vertebrate groups (Montenegro 2004). Additional research is necessary to determine potential reasons for this particular behavior, the role that *collpas* play in the ecology of bat communities in the area, and conservation decisions regarding these vertebrate communities and their resources. Furthermore, analyses of the mineral content of *collpa* water compared to other water sources in southeastern Peruvian Amazon likely will provide useful information to determine the reasons that bats visit and drink water from *collpas*. This study is a first step towards understanding the importance of *collpas* as key resources for frugivorous bats in southeastern Peru, with direct conservation implications for protecting a fully functional forest ecosystem.

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CHAPTER 3. PUDDLES CREATED BY GEOPHAGOUS MAMMALS ARE MINERAL SOURCES FOR FRUGIVOROUS BATS (STENODERMATINAE) IN THE PERUVIAN AMAZON

INTRODUCTION

Natural licks, known locally as *collpas*, appear to be activity hotspots for frugivorous bats in undisturbed forests of Amazonian Peru (Bravo et al. 2008) and Ecuador (Reid et al. 2002, Voigt et al. 2007). *Collpas* are unique places in these forests where several species of geophagous non-volant mammals and birds consume soils (Emmons and Stark 1979, Klaus and Schmid 1998, Krishnamani and Mahaney 2000, Brightsmith and Aramburú 2004, Montenegro 2004, Tobler 2008) and where several species of frugivorous bats often congregate at night to drink water that has collected in puddles or pools (Reid et al. 2002, Voigt et al. 2007, Bravo et al. 2008). Bats drink on the wing, and do not visit dry *collpas* or land on any substrate during their visits. *Collpas* are also called clay licks (Gilardi et al. 1999, Brightsmith and Aramburú 2004), mineral licks (Emmons and Stark 1979, Voigt et al. 2007), and *saladeros* (Reid et al. 2002). Here, I prefer calling them by their local Quechua name—*collpas*—because it does not imply an *a priori* assumption of their function (*e.g.*, providing clay, mineral nutrients, or other resources to the vertebrates that visit them).

A few other records of *collpas* or *collpa*-like pools attracting congregations of bats exist. For instance, Tuttle (1974) reported large numbers of stenodermatine bats drinking from water holes that were frequently visited by tapirs in the Venezuelan Amazon. Ascorra and Wilson (1991) captured large numbers of *Artibeus jamaicencis* at a "*colpa"* [sic] in northeastern Peru. Ascorra et al. (1996) found clay in fecal samples of *Artibeus obscurus* in southeastern Peru, suggesting that they may have been ingesting *collpa* soil. Despite growing awareness and

documentation of *collpa* visitation by bats, still little is known about its causes and its consequences for Amazonian bats.

Most explanations for geophagy hypothesize that the intentional consumption of soil is driven by high concentrations of key resources in unique places, such as *collpas* (Emmons and Stark 1979, Gilardi et al. 1999, Brightsmith and Aramburú 2004, Brightsmith et al. 2008). Most soils in the Amazon are relatively poor in nutrient elements (Stark 1970). Geological stability and high volumes of rain cause leaching of nutrients otherwise made available through litter decomposition (Stark 1970, Jordan and Herrera 1981). As a consequence, plants may contain low amounts of some nutrients (Brightsmith et al. 2008). Therefore, frugivorous species may use *collpas* as reliable secondary sources of nutrients limited in their diets (Gardner 1977, Zortea and Lucena-Mendes 1993, Nelson et al. 2005). Alternatively, bats may use *collpas* as sources of clay itself, for example to help bind plant secondary compounds (Gilardi et al. 1999, Houston et al. 2001). Bats probably do not visit *collpas* to obtain water only (Bravo et al. 2008).

In a previous study in the Peruvian Amazon, Bravo et al. (2008) reported patterns of *collpa* visitation by bats during a dry season (see Chapter 2). Capture rates at *collpas* were 10 times higher than in the surrounding forest and the vast majority of bats were frugivorous (mostly bats in the sub-family Stenodermatinae). Furthermore, there was a strong female bias among bats at *collpas* compared to bats in the forest, and the majority of those female bats was in reproductive condition. Similar patterns have also been reported in Ecuador (Reid et al. 2002, Voigt et al. 2007). Consequently, potential explanations for *collpa* visitation by bats may be especially related to the nutritional demands of reproductive female frugivorous bats.

Bats face numerous physiological demands during reproduction. Energy and nutritional requirements increase significantly during pregnancy and lactation (Studier and Wilson 1991, Barclay 1994, Bernard and Allen 1996, Bernard and Davidson 1996, Korine et al. 2004, Speakman 2008). Reproductive individuals of the Neotropical frugivorous bat *Artibeus*

jamaicensis increased their daily caloric requirement by ~ 240 %, daily protein requirement by ~ 400 %, and daily water intake by ~ 14 % compared to non-reproductive individuals (Studier and Wilson 1991). Furthermore, although bats seem to usually obtain sufficient amounts of nutrients for maintenance from their diets (Wendeln et al. 2000), because of the increased requirements during reproduction some nutrients may become limited (Studier and Wilson 1991, Barclay and Harder 2003, Speakman 2008). To overcome deficiencies, some bats supplement their diets by consuming floral parts, leaves, pollen, nectar, and/or insects (Gardner 1977, Kunz and Diaz 1995, Ruby et al. 2000, Nelson et al. 2005). Nevertheless, the energetic requirements for flying and searching for food are extremely high (Studier and Wilson 1991, Korine et al. 2004). Hence, the use of other reliable mineral sources, such as *collpas*, may be less costly energetically and consequently preferred.

Collpas may function as sources of a variety of minerals, clay, or other resources. Herein I focus on the key cations for frugivorous bats: calcium, magnesium, potassium, and sodium. Calcium is essential for milk and bone production, but it is usually limited during reproduction (Keeler and Studier 1992, Barclay 1994, Studier and Kunz 1995, Bernard and Allen 1997, Kwiecinski et al. 2003, Booher 2008). Although some frugivorous bat species may consume calcium-rich fruits (Wendeln et al. 2000), the amounts acquired may not be sufficient for reproduction (Barclay and Harder 2003).

Sodium is one of the most important nutrients for animals. It is essential for the physiology of homeostasis, nerve impulses, and muscular function (Michell 1995). Despite its importance, sodium may be one of the most limiting nutrients to vertebrates in the tropics, where leaching depletes it from soil (Stark 1970, Emmons and Stark 1979). Because most plants do not require it, they contain low sodium (Stark 1970, Nagy and Milton 1979, Wendeln et al. 2000), perhaps as a deterrent against herbivory (Morris 1991). Consequently, frugivorous species may face sodium constraints, especially during reproduction (Michell 1995).

As well as calcium and sodium, magnesium and potassium are essential minerals for vertebrates. Magnesium provides strength to bones, facilitates enzyme activity, and is needed for nucleic acid and protein synthesis (Morris 1991). Potassium is important in nerve conduction, in muscular contraction, and in osmotic water balance (Morris 1991). Contrary to calcium and sodium, frugivorous bats seem to obtain adequate amounts of magnesium and potassium from their diets (Nagy and Milton 1979, Wendeln et al. 2000).

Soil consumption by geophagous animals has been described as highly seasonal. Temporal patterns of *collpa* visitation are associated with animal requirements of key resources at specific times of the year (Jones and Hanson 1985, Roze 1989, Brightsmith 2004, Ayotte et al. 2006). In temperate forests of North America, higher mineral lick visitation rates of ungulates were observed during spring and summer, which were associated with a drastic change in diet produced by leaf flush at the beginning of spring and milk production for female individuals at the end of summer (Carbyn 1975, Jones and Hanson 1985, Klaus and Schmid 1998, Ayotte et al. 2006). In the Neotropics, Brightsmith (2004) suggests that seasonality of lick use by parrots is due to changes in diet and reproduction. Similarly, *collpa* visitation by bats may display seasonal patterns associated with sexual or reproductive condition.

In this study, I assessed the potential of *collpas* as sources of important nutrients for reproductive female frugivorous bats in the southeastern Peruvian Amazon. I first extended the study of Bravo et al. (2008) to determine patterns of use of *collpas* by bats across seasons. I compared bat activity, species richness, species composition, sex ratio, and reproductive condition between *collpa* and non-*collpa* sites. Second, I investigated general and seasonal patterns of the chemical properties of *collpa* water by comparing mineral concentrations, pH, and electrical conductivity of *collpa* water used by bats to that of other readily available water sources.

METHODS

This study was conducted between 2005 and 2008 at Los Amigos Conservation Concession located at the confluence of the Madre de Dios and Los Amigos rivers in the Department of Madre de Dios in southeastern Peru (12°30' to 12°36' S and 70°02' to 70°09' W). This private concession protects over 140,000 hectares of Amazonian forest within the Moist Humid Ecological Zone (Holdridge et al. 1971); for a comprehensive overview of the region, see Terborgh (1983). The average annual temperature from 2005 to 2007 ranged from 23.93 to 24.13°C, and average annual rainfall ranged from 2152 to 2682 mm, unevenly distributed between wet (~ October-April) and dry seasons (~ May-September) (Atrium 2008).

Bat Capture and Data Analysis

To determine the effect of season on bat activity, I continued the study by Bravo et al. (2008) presented in Chapter 2. In 2005, I selected three *collpas* along the Los Amigos River for study (*Collpa* 1: 12°32'35" S, 70°04'58" W; *Collpa* 2: 12°30'23" S, 70°08'56" W; *Collpa* 3: 12°27'29" S, 70°15'00" W). They were chosen for similarity among *collpas*, but independence in terms of individual bats visiting them. All were in mature floodplain forest, each at about 1 km from the riverbank. *Collpas* were of similar size (*Collpa* 1: 17.4 x 8.3 m; *Collpa* 2: 18 x 7.6 m; *Collpa* 3: 16.3 x 10. 2 m). Each was separated from the others by at least 8 km. I chose a forest sampling site near each *collpa*. In 2007, I added a natural gap site near each *collpa.* Each forest and gap site was from 300 to 500 m from its spatially associated *collpa*.

From September to November (dry season) 2005, I captured bats at *collpa* and forest site types (Bravo et al. 2008). From July to September (dry season) 2007 and from February to May (wet season) 2008, I captured bats at *collpa*, forest, and gap site types following the same protocol used by Bravo et al. (2008). I used 6 x 2.6-m, 36-mm mesh Japanese mist nets (AFO Banding Supplies, Manomet). Once a month, I captured bats at each of the nine sampling sites

making a total of 27 sampling nights at *collpa* and forest sites and 18 sampling nights at gap sites. Since bat activity is often reduced during bright moonlit nights (Morrison 1978), I avoided opening nets five days prior to and after a full moon. In general, nets were opened at dusk (1730-1745 h) and closed at midnight (2400 h). Due to the large numbers of bats at *collpas*, I opened only one mist net, which captured as many bats as two or three people could comfortably process. On busy nights, to avoid causing unnecessary stress to the bats, I closed and opened the net as many times as needed to limit captures to the numbers that I could efficiently handle. In contrast, at forest and gap site types, I deployed between five and ten mist nets along previously opened lines, and nets were never closed during the ~ 6-h sampling period. At all sites, each net was checked every fifteen minutes. Each bat was taken from a net and placed in a clean cotton bag for transfer to the processing station about 100 m from the nets. Bats were kept in bags for no longer than 30 minutes before being processed. I identified, measured, weighed, marked, and recorded sex and reproductive condition of each captured bat. I used field guides to identify bats to the level of species (Emmons and Feer 1997, Tirira 2007). In addition, each individual bat was marked with a chainball necklace that carried a unique numbered aluminum band (Handley et al. 1991). Bats were released after processing.

I defined bat activity as the number of bats per net per hour, for *collpa*, gap, and forest site types in the wet and dry seasons. For all site types, I used the total open net hours. I compared seasonal bat activity at different site types using a two-way block ANOVA. The linear model treated each *collpa* and its associated forest and gap sites along the Los Amigos River as a block, and seasons and site type (*i.e.*, *collpa*, forest, gap) as factors. To meet the assumptions of the analysis, I square root-transformed bat activity data prior to the analysis. I compared the means among the different factor levels that showed a significant effect in the block analysis with the Tukey's Honest Significant Difference method (Tukey HSD).

I determined species composition and relative abundance distributions of bats captured at *collpa*, gap, and forest site types. I compared distributions between paired site types (*collpa* vs. forest, *collpa* vs. gap, forest vs. gap) with two-sample Kolmogorov-Smirnov tests run in SPSS (SPSS Inc. 1990). In addition, I compared species richness among *collpa*, forest and gap site types using an individual-based rarefaction analysis. This analysis calculated for each site type studied the expected number of bat species for a given number of individuals captured. With this information, I generated accumulation curves for comparison among site types. The analysis was performed using the program PAST (PAleontological STatistics, ver.1.25, ∅. Hammer, D. A. T. Harper and P. D. Ryan, May 18, 2004).

I tested the independence of the proportion of frugivorous bats captured at each site type during the dry and wet seasons with a generalized linear model (GLM) with Poisson distribution. I fitted a saturated model and then tested the effect of the interaction terms by removing from the saturated model the interactions of interest and comparing models with an analysis of deviance that used a Chi-squared test (Crawley 2007). Using the same approach, I also tested the independence of sex and female reproductive condition at each site type during the wet and dry seasons. Finally, using Chi-square goodness-of-fit tests I asked whether the sex ratios and the ratios of reproductive to non-reproductive female bats at each site were 50:50.

Water Sampling and Data Analysis

From July to September 2007, February to April 2008 and July to September 2008, I collected water monthly from the three focal *collpas*, as well as from three creeks and three oxbow lakes near the three focal *collpas* along the Los Amigos River. The total sampling size was 27 water samples from *collpas,* creeks and oxbow lakes, respectively. For the first two sampling periods, I collected water on the same days that I captured bats in the area. The final sampling was after I had finished with bat captures. I used two protocols for sampling water. In 2007, I collected 125

ml of water in acid-rinsed and dry Nalgene bottles. That is, before sampling, each bottle was soaked in an acid water solution (HCl 10%), thoroughly rinsed with distilled water, and air-dried to avoid any contamination. To collect a water sample, I conditioned bottles by collecting and then discarding ~ 100 ml of water from near the water's surface. Then I collected 125 ml into the conditioned bottles, which were kept cold and in a dark place until the analysis. Mineral content analysis of calcium, magnesium, potassium, and sodium of the samples was done by the Laboratory of Water and Soil of the Department of Water Resources and Soil at the University Agraria La Molina (http://www.lamolina.edu.pe) in Lima, Peru using atomic absorption spectrometry (AAS). Concentration of minerals was provided by the lab in parts per million. In 2008, I collected water following the protocol used in 2007, but in addition I filtered ~ 15 ml of each water sample with a 0.45 um sterile Nalgene syringe filter into a sterile centrifuge tube. Filtered samples were placed into the refrigerator until analysis was performed. The Soil Testing and Plant Analysis Laboratory at Louisiana State University Agricultural Center (http://www.lsuagcenter.com) analyzed calcium, magnesium, potassium, and sodium content in water samples using inductively coupled plasma (ICP) spectrometry. All mineral concentrations were reported in parts per million. Finally, in September 2008, I collected samples from the Madre de Dios River and the Los Amigos River for general comparative purposes.

I compared water mineral concentrations among water from three sources (*i.e. collpas*, creeks and oxbow lakes) collected in three sampling periods. Specifically, I compared concentrations of calcium, magnesium, potassium, and sodium among water sources at each sampling period using a one-way block ANOVA with repeated measures for each mineral. The linear model considered each *collpa* and its spatially associated creek and oxbow lake along the Los Amigos River as a block and each water sample taken at different times in each site as a repeated measure. To meet the assumptions of the analysis, I log-transformed the concentration data of all minerals but calcium, which was square root-transformed. I also analyzed the effect of

seasonality on the concentrations of calcium, magnesium, potassium, and sodium at different water sources using only data from the wet and dry season of 2008, to minimize any effect of using different labs for water analysis in different years. For each mineral, I used a two-way randomized block design with repeated measures. The linear model took each *collpa* and its associated creek and oxbow lake along the Los Amigos River as a block, seasons and water sources as factors, and each sample taken at different times as a repeated measure. I square root-transformed concentration data of calcium, and log-transformed concentration data of magnesium, potassium and sodium to meet the assumptions of the tests. After the ANOVA analysis, for all minerals I used a Tukey's Honest Significant Difference method (Tukey HSD) to compare the means among the different values of the factors that showed a significant effect.

Finally, I measured *in situ* water pH and electrical conductivity (EC) each time I collected water at all sites. I used a waterproof pH/EC tester (HI 98129, Hanna Instruments Inc., RI, USA), which provides measurements of electrical conductivity in μS/cm corrected for 25°C degrees. I made three readings of pH and EC each time I collected water and then calculated the average value to be used in the analysis. Prior to calculating the average, I converted pH values to $[H^+]$, using $[H^+] = 10^{(-pH)}$. I compared pH values and EC among *collpas*, creeks, and lakes using a one-way block ANOVA with repeated measures. Prior to the analysis I log-transformed the EC values to meet the assumptions of the ANOVA.

Unless specified, all analyses were performed in R (Crawley 2007, R Development Core Team 2007).

RESULTS

Bats at *Collpas*

I captured 2409 bats in a total sampling effort of 2005 open net hours. With sampling efforts of 167, 1280 and 558 open net hours, I captured 1962, 260 and 187 bats at *collpa*, forest, and gap

site types, respectively. To make a reasonable comparison among site types I standardized these data with a measure of bat activity, *i.e.*, number of captures per open net hour. Regardless of the season ($F_{1, 16} = 0.52$, $P = 0.48$), bat activity at *collpas* was significantly higher than at forest and gap site types ($F_{1, 16} = 316.67$, $P < 0.01$; Appendix 2). Furthermore, there was no significant difference in bat activity between forest and gap site types ($P = 0.99$) (Figure 3.1). On average, more than 10 bats per net per hour were captured at *collpas* compared to less than one bat per net per hour captured in forest and gap site types.

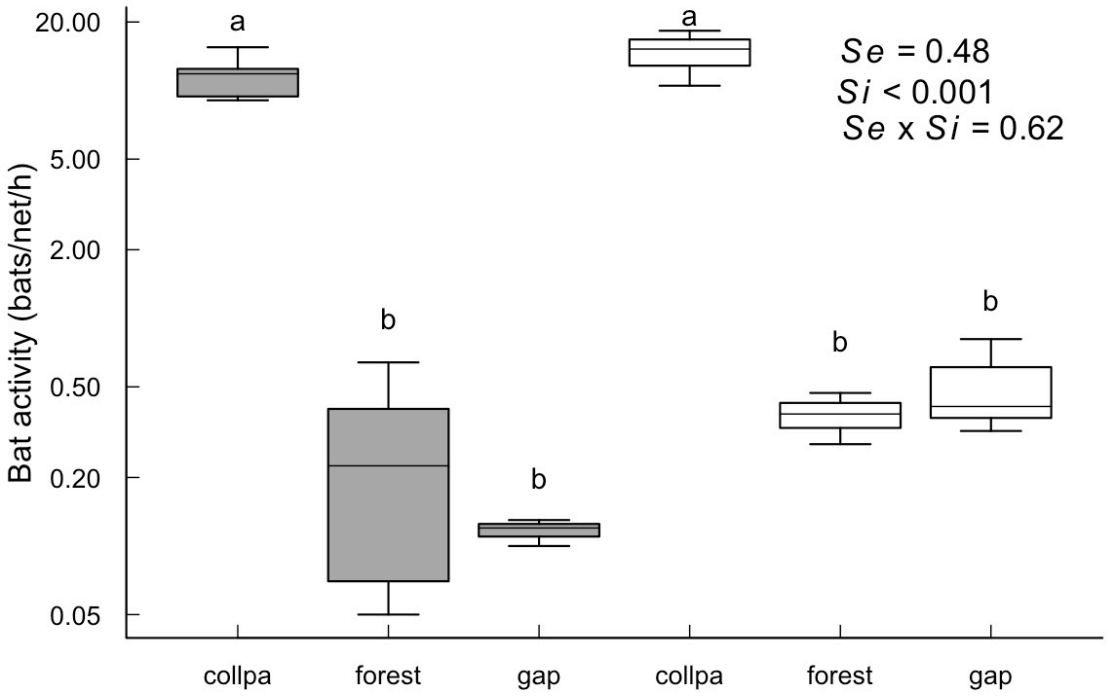


Figure 3.1. Bat activity (bats/net/h) during the dry (gray boxes) and wet seasons (white boxes) at *collpa*, forest and gap site types in southeastern Peruvian Amazon. P values are presented for the season (*Se*) and site type (*Si*) effects. Box plots show the median, upper and lower quartiles, and highest and lowest data values. Note that y-axis is not on a linear scale.

Species Composition and Species Richness

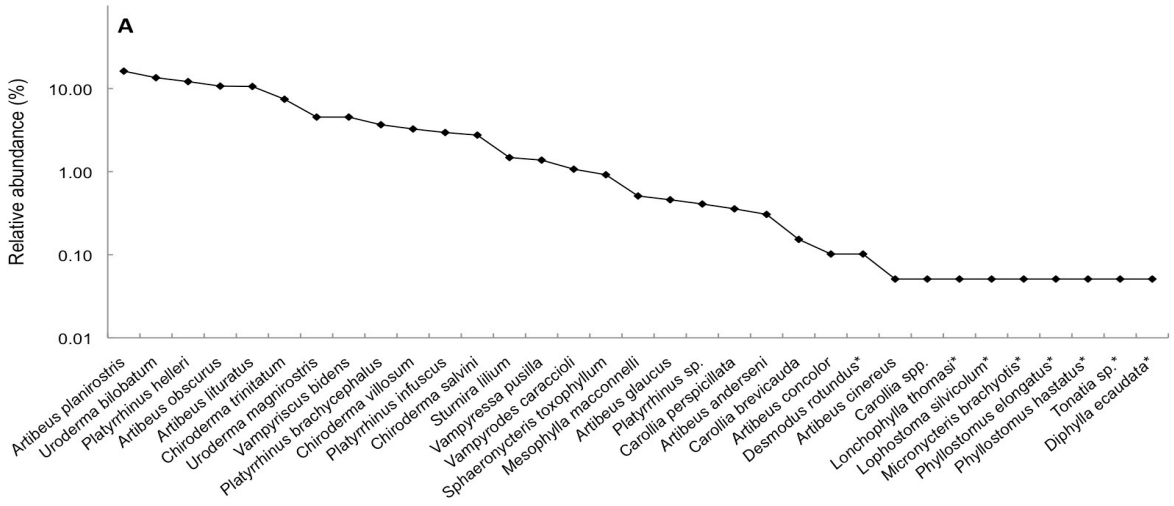
The Kolmogorov-Smirnov analysis showed significant differences in the relative abundance distributions between *collpas* and forests ($Z = 1.50$, $P = 0.02$) and between collpas and gaps ($Z = 2.11$, $P < 0.01$), but there was no significant difference between forests and gaps ($Z = 0.95$, $P = 0.33$) (Figure 3.2). The main difference in species composition among site types was likely caused by differences in Stenodermatinae and Carolliinae. At *collpas*, the five most common species were frugivorous species of the subfamily Stenodermatinae: three large species of the genus *Artibeus*, *Uroderma bilobatum* and *Platyrrhinus helleri*. In contrast, at forest sites and gaps among the most common bats were three Carolliinae species: *Carollia perspicillata*, *C. brevicauda*, and *Rhinophylla pumilio*. Because of the larger numbers of individuals and species registered at *collpas*, more rare species occurred there than at forest sites and gaps. At *collpas*, eighteen species accounted for less than one percent of the total numbers of species captured, whereas at forest sites and gaps seven species at each site type accounted for a similar percentage. Among the rare species at *collpas*, I captured 18 individuals of *Sphaeronycteris toxophyllum*, a very rare species in the forest.

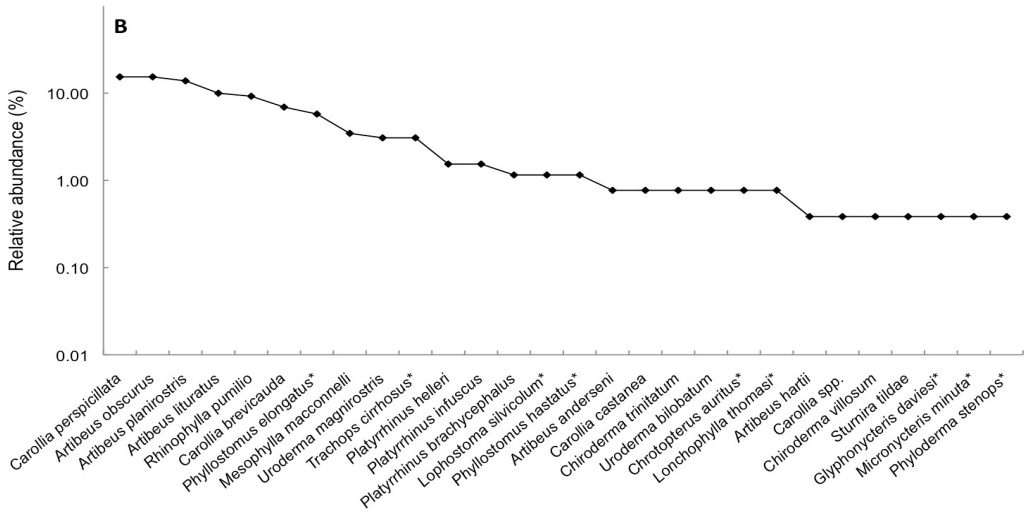
The species richness observed at *collpas* was higher than at forests and gaps. At *collpas*, I identified 34 species, whereas at forest sites and gaps I identified 28 and 30 species, respectively. The accumulation curves showed a higher expected species richness for a given number of individuals at gaps and even higher at forest sites than at *collpas*. Contrary to the forest sites and gaps, the accumulation curve for *collpas* showed an asymptotic shape that indicated that most of the expected species had already been sampled (Figure 3.3).

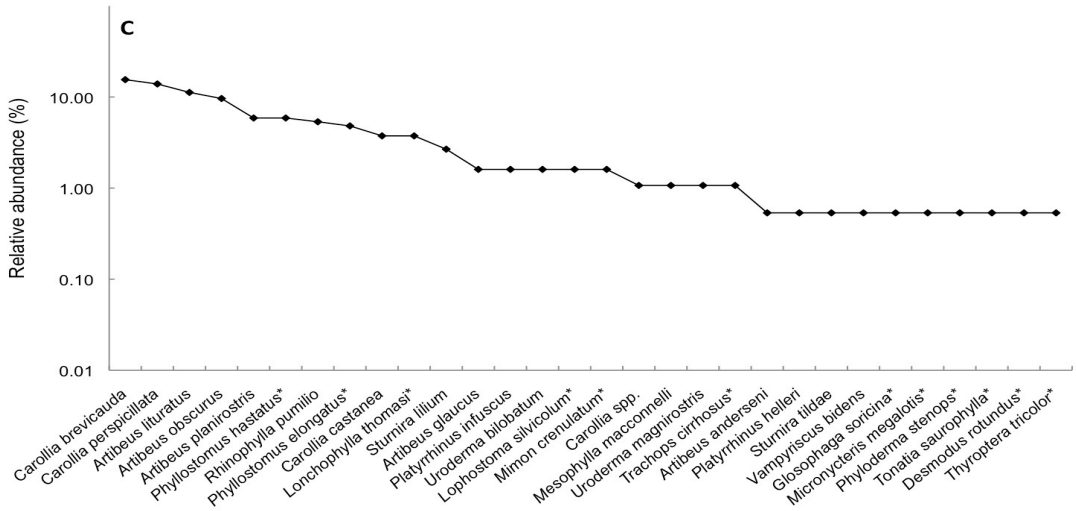
Diet, Sex Ratio, and Reproductive Condition

Regardless of the season, *collpas* were frequently visited by frugivorous bats from the family Phyllostomidae. The proportion of frugivorous and non-frugivorous bats differed among site

Figure 3.2. Species composition and species relative abundance distribution curves for bats at (A) *collpa,* (B) forest, (C) and gap site types in southeastern Peru for all years and seasons combined. An asterisk indicates a non-frugivorous species.


A
Relative abundance (%)
10.00
1.00
0.10
0.01
Artibeus planirostris
Uroderma bilobatum
Platyrrhinus helleri
Artibeus obscurus
Artibeus lituratus
Chiroderma trinitatum
Uroderma magnirostris
Vampyriscus bidens
Platyrrhinus brachycephalus
Chiroderma villosum
Platyrrhinus infuscus
Chiroderma salvini
Sturnira lilium
Vampyressa pusilla
Vampyrodes caraccioli
Sphaeronycteris toxophyllum
Mesophylla macconnelli
Artibeus glaucus
Platyrrhinus sp.
Carollia perspicillata
Artibeus anderseni
Carollia brevicauda
Artibeus concolor
Desmodus rotundus*
Artibeus cinereus
Carollia spp.
Lonchophylla thomasi*
Lophostoma silvicolum*
Micronycteris brachyotis*
Phyllostomus elongatus*
Phyllostomus hastatus*
Tonatia sp.*
Diphylla ecaudata*


B
Relative abundance (%)
10.00
1.00
0.10
0.01
Carollia perspicillata
Artibeus obscurus
Artibeus planirostris
Artibeus lituratus
Rhinophylla pumilio
Carollia brevicauda
Phyllostomus elongatus*
Mesophylla macconnelli
Uroderma magnirostris
Trachops cirrhosus*
Platyrrhinus helleri
Platyrrhinus infuscus
Platyrrhinus brachycephalus
Lophostoma silvicolum*
Phyllostomus hastatus*
Artibeus anderseni
Carollia castanea
Chiroderma trinitatum
Uroderma bilobatum
Chrotopterus auritus*
Lonchophylla thomasi*
Artibeus hartii
Carollia spp.
Chiroderma villosum
Sturnira tildae
Glyphonycteris daviesi*
Micronycteris minuta*
Phyloderma stenops*


C
Relative abundance (%)
10.00
1.00
0.10
0.01
Carollia brevicauda
Carollia perspicillata
Artibeus lituratus
Artibeus obscurus
Artibeus planirostris
Phyllostomus hastatus*
Rhinophylla pumilio
Phyllostomus elongatus*
Carollia castanea
Lonchophylla thomasi*
Sturnira lilium
Artibeus glaucus
Platyrrhinus infuscus
Uroderma bilobatum
Lophostoma silvicolum*
Mimon crenulatum*
Carollia spp.
Mesophylla macconnelli
Uroderma magnirostris
Trachops cirrhosus*
Artibeus anderseni
Platyrrhinus helleri
Sturnira tildae
Vampyriscus bidens
Glossophaga soricina*
Micronycteris megalotis*
Phyloderma stenops*
Tonatia saurophylla*
Desmodus rotundus*
Thyroptera tricolor*

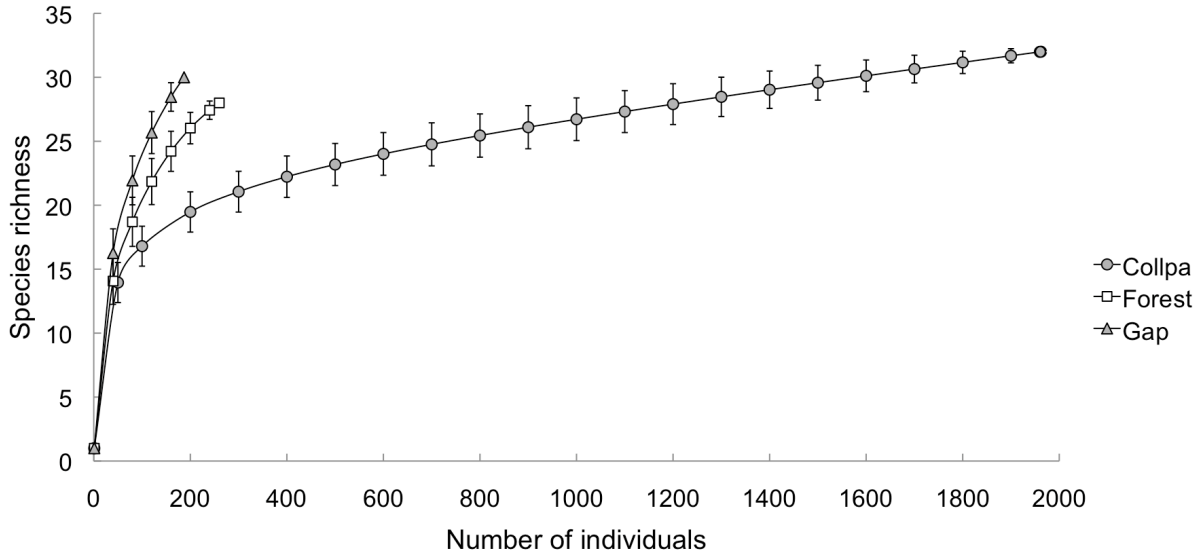


Figure 3.3. Rarefaction curves for bats at *collpa*, forest, and gap site types in southeastern Peru for all years and seasons combined. Vertical lines are ± SD.

types ($P < 0.01$), but not across seasons ($P = 0.86$). Less than one percent of bats captured at *collpas* belonged to a different feeding guild than frugivores, whereas at forests and gaps frugivores represented 65 and 80 percent of the total numbers of captured bats, respectively. Furthermore, most frugivorous bats that visited *collpas* belonged to the subfamily Stenodermatinae. Less than one percent of the individuals of frugivorous species belonged to the subfamily Carolliinae, whereas at forest and gap sites they represented 33 and 40 percent, respectively. In contrast to the *collpas* and forest sites, at gaps more than one family of bats was captured (Appendix 3).

Bats' sex ratios differed significantly among capture sites ($P < 0.01$), but not across seasons ($P = 0.37$). There was a consistently strong female sex bias at *collpas* ($X^2 = 150.5$, $P < 0.001$) compared to the forests and gaps, where the proportion of male and female bats was not significantly different from 50:50 ($X^2 = 0.006$, $P = 0.93$ and $X^2 = 0.45$, $P = 0.5$ respectively; Appendix 3). At *collpas*, about 70 percent of the total number of captured bats were female individuals (Figure 3.4A).

Female bats' reproductive condition differed among *collpas*, forests, and gaps ($P < 0.001$), but it was similar across seasons ($P = 0.11$). There was a higher proportion of female reproductive bats at *collpas* ($X^2 = 139.27$, $P < 0.001$), compared to forests where the proportions of reproductive and non-reproductive female bats were similar ($X^2 = 2.97$, $P = 0.08$), and gaps where the proportions of non-reproductive female bats was higher ($X^2 = 18.18$, $P < 0.001$). At *collpas*, 73 percent of the total number of captured female bats was in reproductive condition (Figure 3.4B).

Water Mineral Content

Concentrations of calcium, magnesium, potassium, and sodium differed among water sources in dry and wet seasons of 2007 and 2008 respectively ($P < 0.01$ for all cases; Appendix 2) (Figure 3.5A – 3.5H). In dry season of 2008, concentrations of all minerals ($P < 0.01$) but magnesium ($P = 0.8$) differed among water sources (Figure 3.5I – 3.5L; Appendix 2). In 2007, the concentrations of all minerals were higher at *collpas* than at creeks and oxbow lakes (Figures 3.5A – 3.5D). In contrast, in wet season of 2008 calcium concentration was higher at oxbow lakes than at *collpas* and creeks, whereas magnesium was present in lower concentrations at oxbow lakes than at *collpas* or creeks (Figures 3.5E and 3.5F). Concentrations of potassium and sodium were higher at *collpas* than at other water sources, similar to 2007 (Figures 3.5G and 3.5H). During dry season of 2008, there was no significant difference in concentration of magnesium among water sources (Figure 3.5J). Concentrations of calcium, potassium and sodium were similar to the wet season of 2008 (Figures 3.5I, 3.5K and 3.5L). Mineral concentrations in the Madre de Dios and Los Amigos River were very similar. Calcium, magnesium, potassium, and sodium concentrations were 12.7 and 14.3, 1.9 and 1.7, 1.1 and 1.6, and 2.4 and 2.8 ppm, respectively.

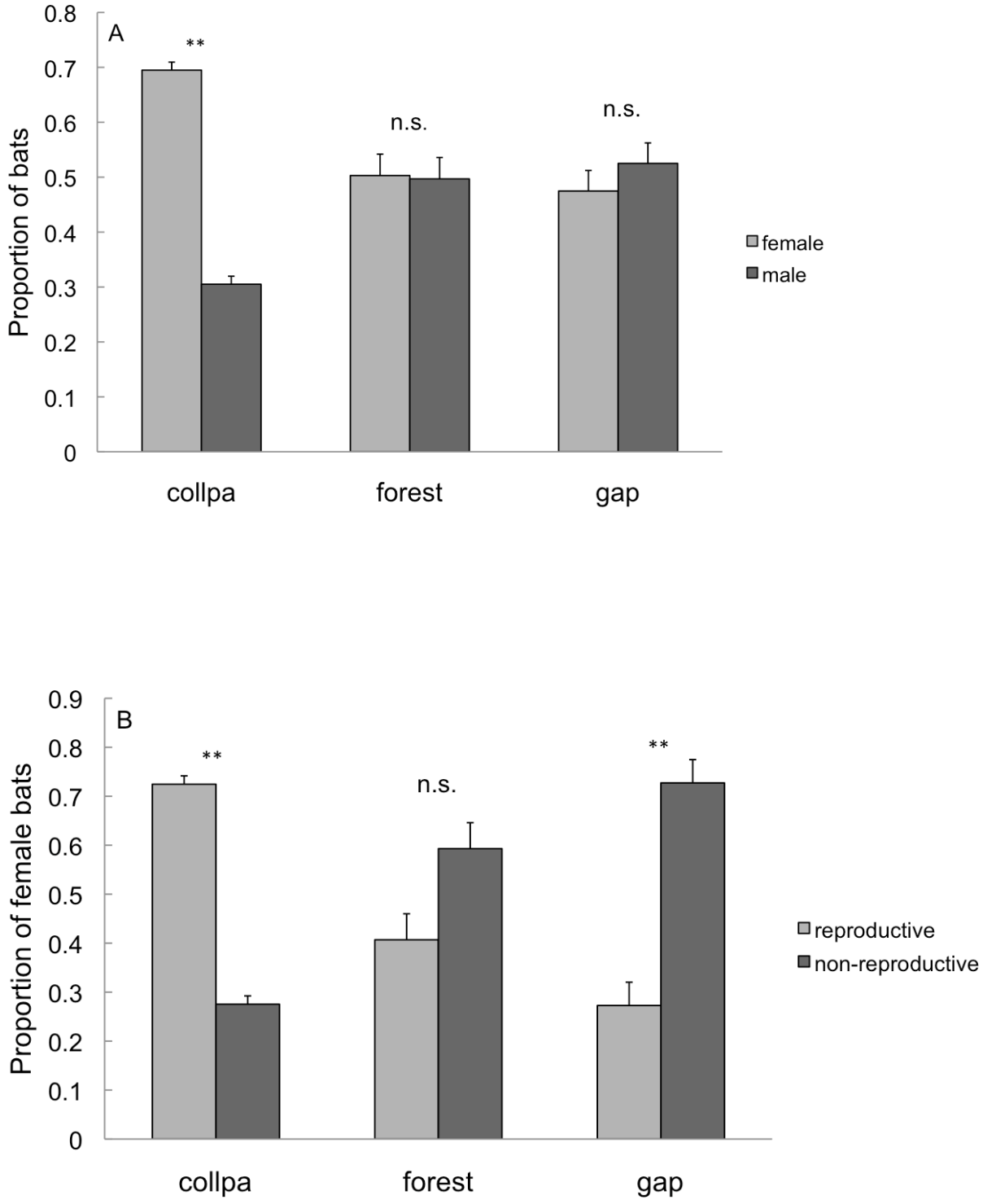


Figure 3.4. Patterns of use of *collpas*, forest sites and gaps by (A) female and male bats and by (B) reproductive and non-reproductive female bats in southeastern Peruvian Amazonia for all years and seasons combined. Error bars are SD from the binomial distribution. An asterisk indicates a significant difference ($P < 0.001$). Asterisks indicate significant differences ($P < 0.001$) and n.s. indicate non-significant differences ($P > 0.05$).

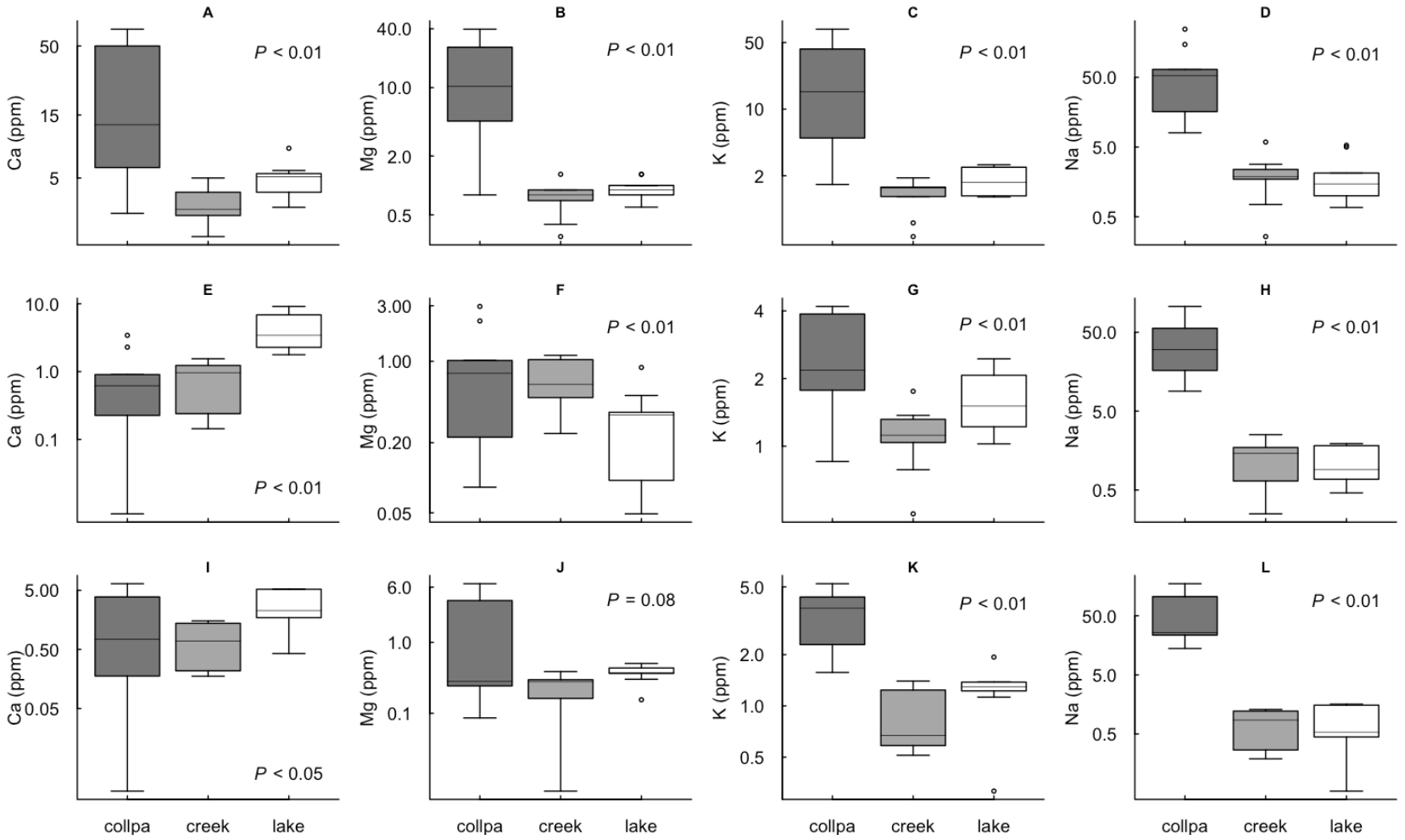


Figure 3.5. Concentrations of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) in parts per million (ppm) for *collpa*, creek, and lake water collected in (A-D) July – September (dry season) 2007, (E-H) February – April (rainy season) 2008, (I-L) and July – September (dry season) 2008. Box plots show the median, upper and lower quartiles, highest and lowest data values, and outliers. Each *P* value is of the one-way ANOVA with repeated measures for each mineral (Appendix 2).

Seasonal Water Mineral Content

In 2008, there was no effect of season (dry or wet) on the concentrations of calcium ($F_{1,40} = 0.08$, $P = 0.77$), magnesium ($F_{1,40} = 0.95$, $P = 0.33$), potassium ($F_{1,40} = 0.22$, $P = 0.27$), and sodium ($F_{1,40} = 2.93$, $P = 0.09$) in *collpas*, creeks and oxbow lakes. However, regardless of season, the concentrations of calcium, magnesium, potassium and sodium differed among water sources ($P < 0.01$ for all minerals; see Appendix 2 for F values). Calcium concentration at oxbow lakes was significantly higher than at *collpas* ($P < 0.01$) and creeks ($P < 0.01$), but the concentrations between *collpas* and creeks were not different from each other ($P = 0.7$). Magnesium concentration at *collpas* was higher than at creeks ($P = 0.03$) and oxbow lakes ($P = 0.01$). The concentrations at creeks and owbow lakes were similar ($P = 0.93$). Potassium concentration was higher at *collpas* than at creeks and oxbow lakes ($P < 0.01$ for both sites), and it was higher at oxbow lakes than creeks ($P = 0.02$). Sodium concentration was significantly higher at *collpas* than at creeks ($P < 0.01$) and lakes ($P < 0.01$), and there was no significant difference between creeks and oxbow lakes ($P = 0.97$).

pH and Electrical Conductivity

There were marginal differences in the pH of *collpa,* creek and oxbow lake water ($F_{2,6} = 4.99$, $P = 0.05$, Figure 3.6A; Appendix 2). *Collpa* water had higher pH than creeks ($P < 0.01$) and oxbow lakes ($P < 0.01$), but creeks and oxbow lakes had similar pH ($P = 0.1$). On average, *collpa* water had 7.48 of pH, whereas creeks and oxbow lakes had 6.12 and 6.45, respectively.

In terms of electrical conductivity, there were highly significant differences among *collpas*, creeks and oxbow lakes ($F_{2,6} = 73.17$, $P < 0.001$, Figure 3.6B; Appendix 2). The electrical conductivity of *collpa* water was significantly higher than in creeks ($P < 0.01$) and oxbow lakes ($P < 0.01$), but the values were similar between creeks and oxbow lakes ($P = 0.74$).

On average, *collpa* water had 290.90 μS/cm compared to 18.95 and 38.62 μS/cm at creeks and oxbow lakes.

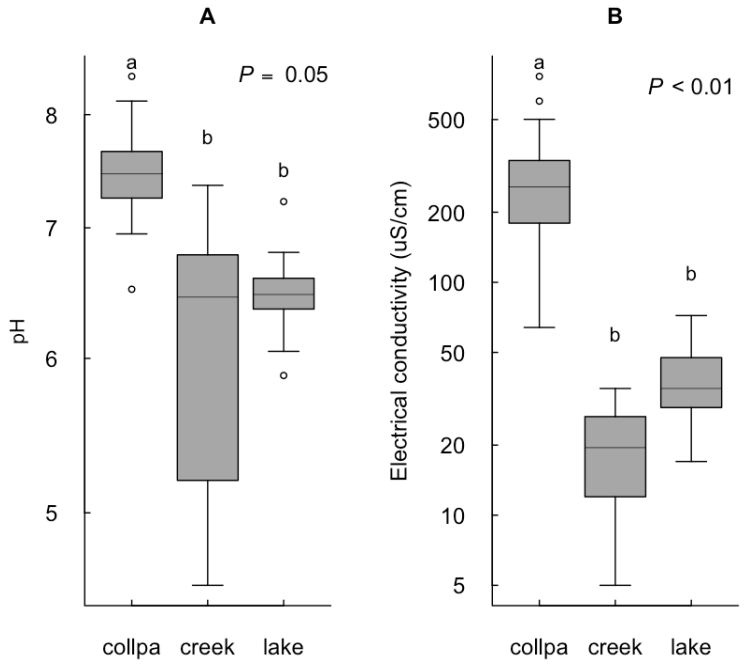


Figure 3.6. (A) pH values and (B) electrical conductivity (EC) (in micro Siemens/cm) at *collpa*, creek, and lake water in the southeastern Peruvian Amazon. Different letters between treatments indicate significant differences and same letters between them indicate no significant differences.

DISCUSSION

Collpas and Bats

This study confirms that regardless of the season, *collpas* are activity hotspots for frugivorous bats in the southeastern Peruvian Amazon, congruent with results from a previous study in a single dry season (Bravo et al. 2008). Hundreds of individuals of several species of frugivorous bats visit *collpas* very consistently over time. Although the sampling effort at forest and gap site types was ~ 8 and ~ 4 times greater than at *collpas*, respectively, the total abundance of bats at *collpas* was 7 to 10 times greater than at non-*collpa* site types. Moreover, the capture rate at *collpas* was over 10 times greater compared to non-*collpa* site types, which is similar to the

results reported by Voigt et al. (2007) and Bravo et al. (2008). In general, frugivorous species are expected to be common in Neotropical assemblages (Ascorra et al. 1996). At *collpas* they were exceptionally predominant. Ninety-nine percent of bats (1953 out of 1962 individuals) were frugivores, compared to 85 and 78 percent at forests and gaps, respectively. In addition, the total species richness and the asymptotic shape of the accumulation curve for *collpas* compared to non-*collpa* site types showed that in effect only a sub-set of the whole community of bats is visiting *collpas*, which as shown by the species composition analysis is mostly composed of frugivorous species. These patterns clearly suggest that *collpa* visitation is strongly related to frugivory.

Even though *collpas* were visited almost exclusively by frugivorous species, the presence of species of the subfamily Carolliinae was much lower than expected. Ninety-eight percent of bats (1942 individuals) captured at *collpas* belonged to the 22 stenodermatines expected in southeastern Peru, and although carolliine species are very common in this region (Ascorra et al. 1996, Voss and Emmons 1996), they accounted for less than one percent (11 individuals) of all bats captured at *collpas*. In striking contrast, carolliine species such as *Carollia brevicauda* and *C. perspicillata* were among the most common species captured at forest and gap site types (Figure 3.2). Therefore, *collpa* visitation seems to be strongly associated to stenodermatine bats. Furthermore, stenodermatine bats are classified as fig-specialists, whereas carolliine species are classified as *Piper* specialists (Fleming 1988, Giannini and Kalko 2004). Thus, *collpa* visitation may not be related to frugivory in general, but to particular diets (Bravo et al. 2008).

This study across seasons allowed us to confirm that *collpa* visitation by frugivorous bats is strongly female biased and, in particular, to reproductive females. Some studies conducted during short periods of time have shown these patterns (Reid et al. 2002, Voigt et al. 2007, Bravo et al. 2008). Here I show the consistency of these patterns across seasons, which strengthens the

argument for the importance of *collpas* for reproductive female frugivorous bats in southeastern Peru.

Minerals in *Collpa* Water

Collpas are mineral-rich water sources. As with most places that attract geophagous animals, *collpas* are particular places in the landscape where some minerals are found in high concentrations (Emmons and Stark 1979, Jones and Hanson 1985, Mokhtar et al. 1990, Klaus et al. 1998, Holdϕ et al. 2002, Brightsmith and Aramburú 2004). Our results show that *collpa* water often contains higher concentrations of selected minerals compared to creeks and oxbow lakes in the southeastern Peruvian Amazon. Furthermore, pH and EC values indicated that water is more basic and has higher concentrations of dissolved salts at *collpas* compared to creeks and oxbow lakes. Although *collpas* contain enriched water, concentrations of some minerals varied from one year to another. In 2007, *collpas* showed a consistently higher concentration of calcium, magnesium, potassium, and sodium compared to creeks and oxbow lakes. This pattern changed in 2008 for calcium and magnesium. Calcium became less concentrated at *collpas* and creeks and more concentrated at oxbow lakes, whereas magnesium concentration was not higher at *collpas* than in the two other water sources. Even though I found some differences in the mineral concentration patterns between years, I did not find evidence to suggest that there was an effect of seasonality on the concentrations of minerals at different water sources for samples taken in 2008. The main effect on mineral concentrations was from water source. Calcium was consistently higher at oxbow lakes than at *collpas* and creeks, whereas potassium and especially sodium were consistently higher at *collpas* than at other water sources for both seasons. Despite the differences found for some mineral concentrations between years, I wish to highlight the consistency of patterns and concentrations of sodium across seasons and years. In general, the levels of mineral concentrations found at *collpas* were much higher in 2007 than in 2008, except

for sodium. For instance, the maximum concentrations recorded at *collpas* in 2007 for calcium and potassium were 67 and 69 ppm, whereas in 2008 they were < 10 ppm. These differences might be explained by the use of different laboratories to conduct the analyses; by the effect of natural disturbances to *collpas* such as the mechanical input of *collpa* soil into the water produced by the presence of large geophagous mammals (e.g. large herds of peccaries or tapirs) previous to the water collection; or by the effect of weather (drought/wet year). To understand the dynamics of minerals at *collpas*, I recommend long-term studies.

Collpa-Water Minerals and Bats

The intriguing question that still remains to be clearly answered is why frugivorous bats visit *collpas*. A set of hypotheses has been proposed to explain the intentional consumption of soil by geophagous non-volant mammals and birds. Soil may provide limited minerals (Brightsmith et al. 2008), antacids (Davies and Baillie 1988), antidiarrheal components (Mahaney et al. 1995), or clay for binding potential dietary toxins (Gilardi et al. 1999). These hypotheses are not mutually exclusive, thus more than one can explain geophagous behavior (Brightsmith et al. 2008). Although bats do not strictly eat soil, the same hypotheses may explain their deliberate water consumption from *collpas.* Even so, our results clearly show that *collpa* water consumed by bats contains high concentrations of minerals that are limited in other water sources in the region.

During reproduction bats face numerous physiological constraints. To overcome those limitations, bats seem to use different mechanisms. First of all, bats have a very consistent life-history trait: small litter sizes in relation to their body mass (Barclay and Harder 2003, Speakman 2008). Moreover, bats increase the amount of food ingested during reproduction (Korine et al. 2004, Speakman 2008), but doing so can be highly expensive energetically (Studier and Wilson 1991, Korine et al. 2004). Thus, other strategies may be energetically less costly than higher food ingestion and consequently practiced. Among insectivorous bat species, calcium limitation during reproduction seems very common because of their low-calcium diets (Keeler and Studier

1992, Barclay 1994, Studier and Kunz 1995, Bernard and Allen 1997, Kwiecinski et al. 2003, Booher 2008). To overcome this constraint many species mobilize calcium from their bones for the development of the fetus and milk production (Kwiecinski et al. 1987, Hood et al. 2006), and others use secondary sources of calcium. Adams et al. (2003) reported large numbers of reproductive female insectivorous bats visiting calcium-rich water pools compared to other calcium-poor water pools, which may be a potential explanation for the phenomenon observed in southeastern Peru. *Collpa* water may provide calcium to female reproductive frugivorous bats. However, whereas many insectivorous species may have calcium-limited diets (Barclay 1994, Bernard and Allen 1997), frugivorous species may consume figs and other wild fruits that contain high concentrations of calcium (Nagy and Milton 1979, Oftedal et al. 1991, Wendeln et al. 2000). Also, other species have been recorded practicing folivory of calcium-rich plant species (Ruby et al. 2000, Nelson et al. 2005). But during reproduction calcium requirements for frugivorous bats increase significantly and the amount obtained from fruits may not be sufficient to cover that demand and thus other sources may be used (Barclay and Harder 2003, Iudica and Bonaccorso 2003). For bats in the Peruvian Amazon, considering the fruit calcium content (Nagy and Milton 1979, Oftedal et al. 1991, Wendeln et al. 2000), the results of calcium concentrations in different water sources and the patterns of bat activity at *collpas* presented in this study, I suggest that calcium supplementation is not the main cause for frugivorous bats to drink *collpa* water. Specifically, changes in calcium concentrations at *collpas* between years were not associated with a change in the activity of bats at *collpas* (Figures 3.1 and 3.5). Furthermore, the concentrations of calcium in the Madre de Dios (12.7 ppm) and Los Amigos rivers (14.27 ppm) were higher than at any other water source, as also shown by Hamilton et al. (2007; who report 18.1+/-0.49 ppm for the Madre de Dios River). Thus, if calcium was limited in the diets of frugivorous bats, *collpas* would not be the best secondary source of it. If concentrations of calcium in fruits consumed by bats were similar to those reported for Central America, it is very

likely that frugivorous bats that visit *collpas* in the Peruvian Amazon may obtain the amounts of required calcium from their diets. However, to have a conclusive explanation in terms of calcium I highly recommend fruit analysis of species consumed by bats that visit *collpas* in the Peruvian Amazon.

Contrary to the patterns observed for calcium, sodium showed a very consistent pattern between years and across seasons. Its total average concentration at *collpas* was more than 30 times higher than that of creeks and lakes. Furthermore, when sodium concentrations at *collpas* are compared to concentrations in the Madre de Dios and Los Amigos rivers, and to the results provided by Hamilton et al. (2007) for the Madre de Dios River and other water sources, it is evident that *collpas* are sodium-rich water sources in the Peruvian Amazon.

Sodium is very limiting to vertebrates in the Neotropics (Stark 1970, Emmons and Stark 1979). Abundant precipitation in the Amazon Basin causes leaching of sodium and as a consequence plants may become sodium deficient, and as most plants do not require sodium, they do not accumulate it (Stark 1970). In that context, frugivorous and folivorous species with low-sodium diets may face sodium limitations, especially for females in reproduction. Contrary to Voigt et al. (2008), who report sodium requirements for growth and reproduction of small mammals at 0.001 ppm of dry matter, the National Research Council (1995) and Dempsey (2004) reports a minimal requirement of 500 and 600 ppm of sodium respectively. In addition, the maintenance requirement, amount of sodium needed to maintain healthy adults except during growth and reproduction (Michell 1995), for a 45-g *Artibeus jamaicencis* was estimated by Studier and Wilson (1991) at 14 mg sodium/animal/day (based on the minimal requirement of 0.6 mmol/day for growing rats provided by Michell 1995). Dry fruits of *F. insipida* contain ~500 ppm of sodium (Nagy and Milton 1979, Studier and Wilson 1991), thus an 8-g fresh fruit (80% moisture) provides about 0.8 mg of sodium. But bats extract ~ 60 % of the fruit juice (Morrison 1980), so the amount of sodium available per fruit is ~ 0.5 mg. Therefore, each bat needs to

consume about 30 fruits/day to meet only the maintenance requirement. This number of fruits is higher than that required to meet the daily caloric and protein needs (Studier and Wilson 1991). Thus, under a low-sodium diet scenario, bats seem to invest extra energy flying and searching for food to meet a sodium requirement that increases during reproduction (Michell 1995).

The limitation of sodium in fruits consumed by stenodermatine bats may drive the consumption of sodium-rich *collpa* water. At *collpas*, most frugivorous bats are stenodermatines, which are known fig-specialists (Kalko et al. 1996, Giannini and Kalko 2004) and many fig species were found to contain low levels of sodium (Nagy and Milton 1979). However, Wendeln et al. (2000) reported some sodium-rich fig species in Panama (e.g. *Ficus glabrata*, with 2800 ppm), and suggested that bats may prefer those species to overcome shortage of this nutrient. To know whether fruits are limited in sodium in the southeastern Peruvian Amazon, I need to determine the mineral content of fruits consumed by stenodermatine bats that visit *collpas*. If *Ficus*-feeding bats' diets are indeed low in sodium in southeastern Peru, the consistency of sodium concentrations among water sources and the consistency in the patterns of *collpa* visitation by female frugivorous species over time would clearly suggest that *collpas* may be important secondary sources of sodium in the Peruvian Amazon in the same way clay licks or minerals licks have been suggested as sodium sources for geophagous animals worldwide (Emmons and Stark 1979, Mokhtar et al. 1990, Klaus and Schmid 1998, Gilardi et al. 1999, Holdϕ et al. 2002, Brightsmith and Aramburú 2004, Ayotte et al. 2006, Brightsmith et al. 2008).

Ultimately, more than one mineral could be provided by *collpas.* Potassium and magnesium do not seem to be limited in fruits (Nagy and Milton 1979, Wendeln et al. 2000). However, during reproductive periods (pregnancy and lactation) bats increase their demands for all nutrients. Thus, even for those minerals that are available in fruits consumed by bats, *collpas* may function as reliable sources in an otherwise nutrient-poor landscape.

In conclusion, *collpas* are activity hotspots for frugivorous bats in the southeastern Peruvian Amazon. Hundreds of female reproductive stenodermatine bats visit individual *collpas* very consistently over time. A likely explanation for this behavior is that *collpa* water provides limiting minerals. Higher concentrations of sodium in *collpa* water compared to other minerals and other water sources suggest that frugivorous bats may be using *collpas* as secondary sources of sodium, especially during reproduction when there is an increase in the demand for nutrients. In a similar way, Emmons and Stark (1979), Gilardi et al. (1999), Brightsmith and Aramburú (2004), and Brightsmith et al. (2008) suggested that non-volant mammals and birds in the southeastern Peruvian Amazon are driven to *collpas* because of the higher concentrations of sodium in the soil. To better support this hypothesis, I need to determine the mineral content of fruits consumed by stenodermatine bats that visit *collpas* in large numbers, as well as from carolliine bats that are less common. This information will allow me to estimate the extent of mineral limitations in bats' diets and the role of *collpas* as mineral sources. Although I do not address the hypothesis that *collpas* are sources of clay that may potentially bind secondary metabolites from bats' diets as suggested by Voigt et al. (2008), because of the low content of tannins in most ripe figs consumed by bats (Wendeln et al. 2000), the benefits of neutralization of toxins is not a likely explanation for bat visitation to *collpas*. Thus, I maintain that mineral supplementation is a more plausible explanation for this phenomenon. I recommend more experimental studies; meanwhile based on the present studies, I recommend *collpas* to be considered as important conservation targets in the Peruvian Amazon.

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CHAPTER 4. TESTING UNDERLYING CAUSES FOR *COLLPA* VISITATION BY FRUGIVOROUS BATS (PHYLLOSTOMIDAE): MINERAL CONTENT OF FRUITS CONSUMED BY BATS THAT FREQUENTLY VISIT *COLLPAS* (STENODERMATINAE) AND THOSE THAT DO NOT (CAROLLIINAE) IN THE PERUVIAN AMAZON

INTRODUCTION

Collpas are open places in the forest where geophagous animals eat soil (Emmons and Stark 1979, Terborgh 1983, Gilardi et al. 1999, Reid et al. 2002, Brightsmith and Aramburú 2004, Montenegro 2004, Tobler 2008). In addition to geophagous animals, large numbers of frugivorous bats (Stenodermatinae), mostly reproductive females, congregate at *collpas* to drink mineral-rich water (Bravo et al. 2008, Chapter 3). *Collpa* water contains high concentrations of selected minerals, especially sodium (Chapter 3). Thus, *collpas* may function as mineral sources for reproductive female frugivorous bats.

Fruits that frugivorous bats consume vary in quality of nutrients and energy required for maintenance and reproduction (Dumont 2003). Most members of the sub-family Stenodermatinae feed primarily on *Ficus* species (Ascorra et al. 1996, Kalko et al. 1996, Wendeln et al. 2000, Giannini and Kalko 2004), whereas most species from the sub-family Carolliinae feed mainly on *Piper* species (Fleming 1988, Ascorra et al. 1996, Giannini and Kalko 2000). *Ficus* species often produce low-quality fruits (Morrison 1980, Herbst 1986, Dumont 2003). They usually contain less nitrogen and lipid concentrations and more indigestible fiber than *Piper* fruits (Fleming 1988, Dumont 2003). However, Wendeln et al. (2000) found substantial variation in the concentrations of protein (nitrogen) and lipids in 14 *Ficus* species on Barro Colorado, Panama. Hence, they suggested that bats may obtain proteins and lipids needed by feeding on different species of figs.

Other studies showed that *Ficus* fruits contain high concentrations of essential minerals for bats. For instance, in the tropics, figs are considered important sources of calcium (Nagy and Milton 1979, Gilardi 1996, O'Brien et al. 1998, Wendeln et al. 2000). Contrary to calcium, patterns of sodium concentrations vary among studies. Wendeln et al. (2000) reported an average sodium concentration of 1690 ppm (1050 – 2800 ppm) for 14 fig species from Barro Colorado, Panama. In contrast, Gilardi (1996) reported an average value of 29.86 ± 21.02 ppm for eight species from Manu National Park and Tambopata National Reserve, Peru. Thus, the likelihood of sodium limitation in fig specialists may vary across the tropics.

Piper fruits consumed by *Carollia* species appear to contain high concentrations of some minerals. Studier et al. (1995) reported high concentrations of calcium and sodium for *Piper* species from the northeastern Peruvian Amazon (15,260 ppm and 730 ppm respectively). *Carollia* species in the region could meet their basic calcium and sodium needs from their *Piper* diet.

However, reproduction in bats involves high physiological costs (Barclay 1994, Korine et al. 2004, Hood et al. 2006, Speakman 2008). During the reproductive cycle, there is an increase in the demand for nutrients and energy (Studier and Wilson 1991, Barclay 1994, Korine et al. 2004, Speakman 2008). For instance, because calcium is used to produce milk and to develop bats' offspring's bones, its daily requirement increases significantly during the reproductive period (Barclay 1994, Hood et al. 2006, Speakman 2008). In addition, bats are weaned when they have nearly reached their adult sizes (81% *Artibeus jamaicencis*; Kwiecinski et al. 2003; and 88% for *Carollia perspicillata*; Barclay 1994). Thus, their calcium demands are high for a long period of time (> 60 days; Kwiecinski et al. 2003). Also, an increase in the requirements of other minerals, such as magnesium and sodium, has been reported (Studier and Wilson 1991, Michell 1995, National Research Council 1995).

Bats' life histories, as well as behavioral and ecological traits, may reduce some physiological constraints during reproduction. Bats have small litter sizes relative to their body sizes (Barclay 1994, Barclay and Harder 2003). Also, reproductive bats increase the amount of food ingested compared to non-reproductive bats (Korine et al. 2004). However, flying to obtain more food can be energetically costly (Barclay 1994, Korine et al. 2004), particularly when the quality of food is low and large amounts of it are needed to reach minimal nutrient requirements. Thus, secondary sources that offer nutrients and/or energy at a lower cost may be preferred.

Several bat species consume secondary items that may provide limited resources in their diets (Gardner 1977, Zortea and Lucena-Mendez 1993, Kunz and Diaz 1995, Ruby et al. 2000, Nelson et al. 2005). Some frugivorous species consume leaves, floral parts, pollen, or insects (Gardner 1977, Kunz and Diaz 1995, Ruby et al. 2000, Nelson et al. 2005). Other species use mineral-rich water sources (Adams et al. 2003, Iudica and Bonaccorso 2003). For example, in Colorado USA, large numbers of female reproductive insectivorous bats supplement their calcium intake by drinking water from calcium-rich puddles (Adams et al. 2003).

In this study I assessed the hypothesis that key minerals are limited in some bats' diets in southeastern Peru. If certain minerals are limited in the diets of specific frugivorous bats, it is likely that those bats use secondary sources, such as *collpas,* to overcome deficiencies. Because *collpas* are visited mostly by frugivorous bats of the sub-family Stenodermatinae (which are routinely found to be *Ficus* specialists), and rarely by frugivorous bats of the sub-family Carolliinae (which are most often found to be *Piper* specialists), I determined whether bats captured at *collpas* had a different diet than bats captured at non-*collpa* sites. For doing so, I explored the diet composition of twenty-two bat species and then assessed whether bat species commonly found at *collpas* had a specific diet type compared to bats at non-*collpa* site types. In addition, I examined patterns of minerals and nitrogen content, as a measure of protein, for *Ficus*

and *Piper* fruits. Finally I determined and compared the composition of four selected minerals and nitrogen between the two focal genera of fruits.

METHODS

Study Site

I conducted this study at Los Amigos Conservation Concession in the Department of Madre de Dios, in southeastern Peru. This private concession is located in the confluence of the Madre de Dios and the Los Amigos Rivers and protects over 140,000 ha of lowland tropical forest classified within the Moist Humid Ecological Zone (Holdridge et al. 1971). Average annual temperature from 2005 to 2007 ranged from 23.93 to 24.13°C, and average annual rainfall ranged from 2152 to 2682 mm, unevenly distributed between wet (~ October-April) and dry seasons (~ May-September) (Atrium 2008).

Fecal Sample Collection and Analysis

To determine whether species commonly found at *collpas* have a specific diet type compared to bats found in other site types, between September to November 2005, July to September 2007, and February to April 2008, I collected fecal samples from bats captured at *collpa,* forest and gap site types (Chapter 2 and 3). I selected three relatively independent focal *collpas* along the Los Amigos River, each of which was paired with a forest site located at about 300 to 500 m. To account for canopy openness similar to *collpas*, I associated a gap site to each *collpa*-forest pair in 2007. I captured bats using 6-m mist nets. At *collpas,* I used a single mist net that captured the number of bats that was comfortably processed by two to three people. At gap and forest site types I deployed between 6-10 mist nets along previously opened trails. I opened the nets from dusk (1730 h -1745 h) to midnight (2400 h). At *collpas,* I opened and closed the net, depending on the number of bats captured. A more detailed explanation of the methods used to capture bats at each site type is found in Bravo et al. (2008) and Chapter 3. After each bat was processed, I

collected fecal samples from the cotton bag where it was temporarily kept. I placed each fresh sample on a filter-paper disk of diameter 5.5 cm and then placed the folded filter paper into a coin envelope labeled with the bat species and capture site. To avoid sample deterioration, after drying them using a conventional oven, I kept them in a sealed container with silica gel until analysis. Using a stereoscope I analyzed the content of each sample. I identified most of the seeds at least to the genus level. Because of the similarity among seeds of *Ficus* species, I was not able to identify them to the species level. I classified *Piper* as morphotypes, using the sizes and shapes of the seeds and comparing them to a reference collection I made. Also, I identified three species of *Cecropia*, comparing seeds to a reference collection. Seeds of the families Araceae, Clusiaceae, Cucurbitaceae and Solanaceae were identified based on their seed shape. An experienced field botanist at the site, Fernando Cornejo, helped in the identification process.

Items identified in the fecal samples were classified into four categories: seeds, pulp, soil, and insects. Seeds were classified as members of the genera *Cecropia* (Moraceae)*, Ficus* (Moraceae)*, Phyllodendron* (Araceae)*, Piper* (Piperaceae)*, Solanum* (Solanaceae) and *Vismia* (Clusiaceae), the family Cucurbitaceae, and undetermined species. For the analysis, I grouped seeds of *Phyllodendron, Solanum, Vismia*, and Cucurbitaceae into a single category because of their small sample sizes. With this classification, I examined the relationship between type of diet and bat species using a Correspondence Analysis (CA) for all fecal samples collected across all site types. In addition, to determine whether there was a preference by certain bat species for *collpas*, I compared the total abundance of each bat species captured among site types (*collpa,* forest, gap) using a Goodness-of-Fit G test (Sokal and Rohlf 1995). I only performed the analysis for species whose expected values were higher than 5 individuals. The α-levels were adjusted using the Bonferroni correction method (Gotelli and Allison 2004). Finally, using the results of the two previous analyses I determined whether the most common species found at *collpas* had a preference for a specific type of diet.

Fruit Sampling and Analysis

From February to April 2008 and July to August 2008, I collected ripe fruits from *Ficus* and *Piper* species. Twice a week I systematically walked along the trail system of the Los Amigos Biological Station that covers approximately 50 km in the floodplain and terra firme forest. Each time I identified a fig tree producing ripe fruits, I collected intact ripe fig fruits from the ground. I also collected leaves and recorded general characteristics of the tree for its identification. *Piper* species are generally shrubs (Gentry and Vasquez 1993), thus ripe infructescences were collected directly from the plant. I determined the ripeness of each infructescence by it softness. When no ripe infructescences were found, I covered unripe ones with a soft mesh cloth until they became soft. Also, I collected a botanical sample of each plant for identification. I placed fruits in paper envelopes and dried them in a conventional oven at ~ 60 °C for ~ 12-18 hours. When fruits were > 4 g, I cut them in small pieces to accelerate the drying process and to minimize infestation by decomposers. Dry samples were kept in a dry container until the analysis was performed. The Soil Testing and Plant Analysis Laboratory at Louisiana State University Agricultural Center (http://www.lsuagcenter.com) analyzed twelve elements: boron, calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, potassium, sodium, sulfur, and zinc content of the fruit samples (Appendix 4). To a minimum of 0.5 g ground dry plant matter, they added 5 ml concentrated HNO_3 and waited for 50 min. After that, they added 3 ml H_2O_2 and let the sample digest for 2.75 hr on a heat block. Finally, they cooled and diluted the samples to read the concentration of minerals using inductively coupled plasma (ICP) spectrometry. For nitrogen, they used 0.1 g of dry matter to determine the concentration by dry combustion using a Leco carbon-nitrogen (CN) analyzer. Concentrations were provided in parts per hundred (%) for most minerals. Sodium and nitrogen concentrations were provided in parts per million (ppm). For comparative purposes I converted parts per hundred to parts per million when necessary.

Although I was interested in the mineral content of fruits, we also determined the concentration of nitrogen because of its increased demand during reproduction (Studier and Wilson 1991, Speakman 2008). Thus, I explored patterns of mineral and nitrogen content among fruits of *Ficus* and *Piper* with a principal component analysis. In addition, using an *a priori* contrasts analysis of variance (Gotelli and Ellison 2004), I compared the concentrations of four key minerals: calcium, magnesium, potassium, and sodium, as well as nitrogen between *Ficus* and *Piper* species. I employed an adjusted α-level for all contrasts using the Bonferroni correction method. All statistical analyses were performed in R (Crawley 2007, R Development Core Team 2007).

RESULTS

Bats' Diets Composition

I collected a total of 245 fecal samples from bats: 103, 60, and 82 samples from *collpas,* forests and gaps, respectively. These samples came from five, 23, and 44 percent of the total number of bats captured at each site type (1962 at *collpas*, 260 at forest, and 187 at gaps). At *collpas,* samples came from 16 species of bats, whereas at forests and gaps they came from 12 and 10 species, respectively. At *collpas,* bat species were all phyllostomid frugivores, 13 belonged to the subfamily Stenodermatinae and 2 to the subfamily Carolliinae (Table 4.1). At forests and gaps, although all but one bat species belonged to the family Phyllostomidae, they belonged to more than one feeding guild (*i.e.* frugivores, omnivores, and insectivores; Tables 4.2 and 4.3). Samples from *Carollia* species were more common in forest and gap site types than at *collpas.* At *collpas,* samples from carolliine species accounted for less than one percent of the total number of samples, whereas at forest and gap site types they accounted for 63 and 88 percent, respectively (Table 4.1-4.3).

The correspondence analysis shows a clear distinction between stenodermatine and carolliine species (Figure 4.1). Most stenodermatine species clustered as *Ficus* specialists, whereas all

carolliine species clustered towards a more diverse diet, most of it composed of *Piper* fruits (Table 4.1-4.3). *Cecropia* was the main component of the diets of *Artibeus lituratus*, *Phyllostomus hastatus* and *Platyrrhinus brachycephalus.* In addition, fecal samples from five stenodermatine species, *Artibeus planirostris, Chiroderma trinitatum, Chiroderma villosum, Platyrrhinus infuscus* and *Uroderma bilobatum* contained soil.

Table 4.1. Diet composition of bats captured at *collpas* in southeastern Peruvian Amazon.

	seeds				pulp	soil	insects	und.
	Moraceae		Piperaceae	Ara./Clu.				
	Cecropia	*Ficus*	*Piper*					
Family and species								
Phyllostomidae								
Carolliinae								
Carollia brevicauda								2
Carollia perspicillata			3	1				
Stenodermatinae								
Artibeus lituratus	9	2						
Artibeus obscurus	4	12			1			
Artibeus planirostris	1	12			1	2		
Chiroderma salvini		3						1
Chiroderma trinitatum		2				2		
Chiroderma villosum		3			1	1		
Platyrrhinus brachycephalus	4	1						
Platyrrhinus helleri	1	1			1			
Platyrrhinus infuscus	8	4				2		
Sturnira lilium	1		2	1				1
Uroderma bilobatum		8			2	1		
Vampyressa pusilla		1						
Vampyriscus bidens		1						
Total	**28**	**50**	**5**	**2**	**6**	**8**	**0**	**4**

Abbreviations are as follows: Araceae (Ara.), Clusiaceae (Clu.), and undetermined (Und.).

Stenodermatine bats showed a strong preference for *collpas*. Sixteen out of seventeen stenodermatine species analyzed were present in higher proportions at *collpas* than at non-*collpa* site types (Table 4.4). In contrast, *Carollia brevicauda* and *C. perspicillata* were more common in gaps and forests, respectively, compared to *collpas* (Table 4.4). Similarly, *Phyllostomus elongatus* and *Phyllostomus hastatus* were significantly more common at non-*collpa* site types.

Table 4.2. Diet composition of bats captured in forest sites in southeastern Peruvian Amazon.

	seeds				pulp	soil	insects	und.
	Moraceae		Piperaceae	Ara./Clu./ Cuc./Sol.				
	Cecropia	*Ficus*	*Piper*					
Family and species								
Phyllostomidae								
Phyllostominae								
Phylloderma stenops				1				
Phyllostomus elongatus	2						3	
Phyllostomus hastatus	4							
Carolliinae								
Carollia brevicauda	3		6	1	1			
Carollia perspicillata	1		8	1	3		3	1
Rinophylla pumilio			1	6	2		1	
Stenodermatinae								
Artibeus obscurus	1	2	1					
Artibeus planirostris	2	1						
Chiroderma trinitatum		1						
Mesophylla macconnelli					1			
Platyrrhinus infuscus	1							
Sturnira lilium				2				
Total	**14**	**4**	**16**	**11**	**7**	**0**	**7**	**1**

Abbreviations are as follows: Araceae (Ara.), Clusiaceae (Clu.), Cucurbitaceae (Cuc.), Solanaceae (Sol.), and undetermined (Und.).

Table 4.3. Diet composition of bats captured in gaps in southeastern Peruvian Amazon.

	seeds				pulp	soil	insects	und.
	Moraceae		Piperaceae	Ara./Clu./ Sol.				
	Cecropia	*Ficus*	*Piper*					
Family and species								
Phyllostomidae								
Phyllostominae								
Phyllostomus elongatus	1		1		1		1	
Phyllostomus hastatus	2							
Carolliinae								
Carollia brevicauda	2		12	1	3		1	2
Carollia castanea			6	1	1		1	2
Carollia perspicillata	3		19	3			4	4
Rinophylla pumilio	2		2	1			2	
Stenodermatinae								
Artibeus obscurus	1							
Mesophylla macconnelli					1			
Sturnira lilium		1						
Thyropteridae								
Thyroptera tricolor							1	
Total	**11**	**1**	**40**	**6**	**6**	**0**	**10**	**8**

Abbreviations are as follows: Araceae (Ara.), Clusiaceae (Clu.), Solanaceae (Sol.), and undetermined (Und.).

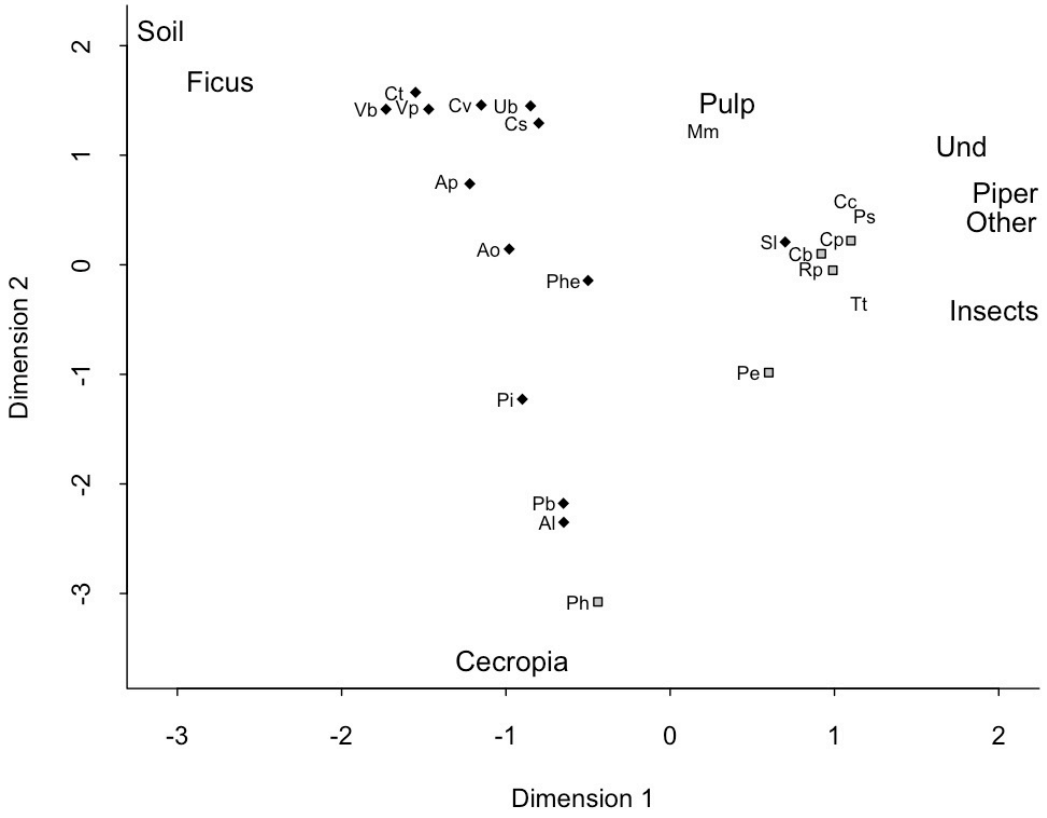

Figure 4.1. Correspondence analysis (CA) of the diet of twenty-two bat species from southeastern Peru. Bat species are abbreviated as *Artibeus lituratus* (Al), *A. obscurus* (Ao), *A. planirostris* (Ap), *Carollia castanea* (Cc), *C. brevicauda* (Cb), *C. perspicillata* (Cp), *Chiroderma salvini* (Cs), *C. trinitatum* (Ct), *C. villosum* (Cv), *Mesophylla macconnelli* (Mm), *Phyllostomus elongatus* (Pe), *P. hastatus* (Ph), *Platyrrhinus brachycephalus* (Pb), *P. helleri* (Phe), *P. infuscus* (Pi), *Phyloderma stenops* (Ps), *Rhinophylla pumilio* (Rp), *Sturnira lilium* (Sl), *Thyroptera tricolor* (Tt), *Uroderma bilobatum* (Ub), *Vampyriscus bidens* (Vb), and *Vampyressa pusilla* (Vp). The "Other" category of diet includes seeds of Araceae, Clusiaceae, Cucurbitaceae, and Solanaceae, and "Und" accounts for undetermined species. A diamond shows bat species overrepresented at *collpas*, whereas a square shows species underrepresented compared to non-*collpa* sites. No symbol accounts for species without significant differences between *collpa* and non-*collpa* sites.

Mineral and Nitrogen Content of *Ficus* Versus *Piper* Species

Ripe fruits from a total of 10 *Ficus* and 6 *Piper* species were collected. *Ficus* and *Piper* species differ in their mineral and nitrogen content (Figure 4.2). The principal component analysis shows

that ~50% of the total variation was explained by the two first components. Principal component 1 (PC1) explained 31%, whereas principal component 2 (PC2) explained 21% (Figure 4.2). The loading values showed that nitrogen and sulfur contributed the most to PC1 (-0.476 and -0.415 respectively), whereas boron and calcium did the same for PC2 (-0.54 and -0.504 respectively; Table 4.5).

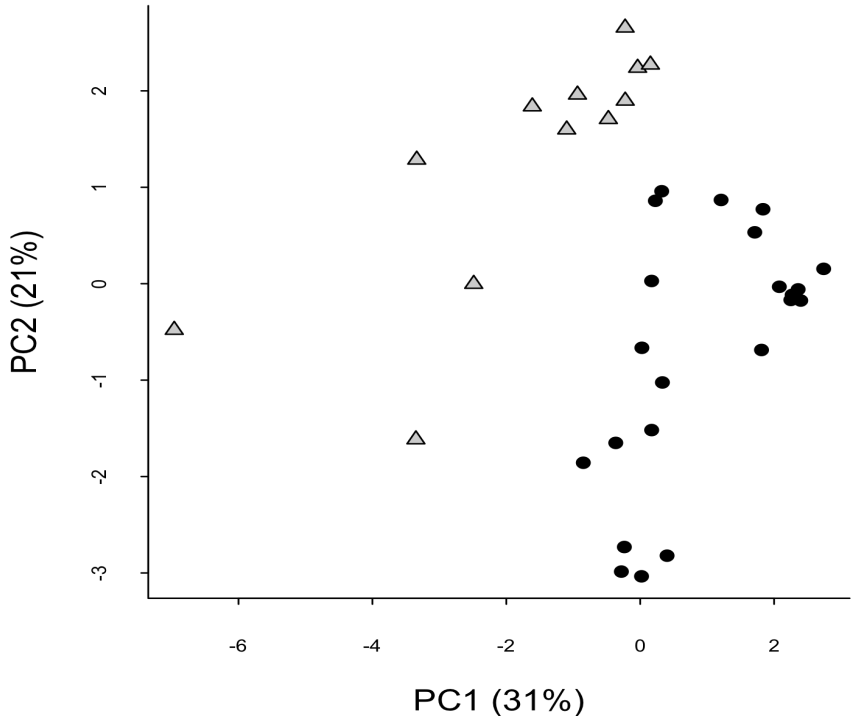

Figure 4.2. Plot for the two first principal components from the PCA analysis of nutrient content of *Ficus* and *Piper* fruits. Each gray triangle represents an individual *Piper* plant, whereas each dark circle represents an individual *Ficus* plant.

Ficus fruits had higher concentrations of calcium and potassium compared to *Piper* fruits (Ca: $t = 22.92$, $P < 0.001$; K: $t = 5.50$, $P < 0.001$). On the other hand, *Piper* fruits had higher concentration of nitrogen compared to *Ficus* fruits ($t = -14.90$, $P < 0.001$). No significant differences were found in the concentrations of magnesium ($t = -1.09$, $P = 0.3$) and sodium ($t = -2.45$, $P = 0.03$; Table 4.6).

Table 4.4. Total abundance of bats captured at *collpas,* forest sites, and gaps, and *G* and *P* values of the goodness-of-fit tests. An asterisk after *P* values indicates significant differences among site types for alpha values corrected by the Bonferroni method.

	Collpa	**Forest**	**Gap**	***G***	***P***
Subfamily and species					
Phyllostomidae					
Phyllostominae					
Chrotopterus auritus		2			
Glyphonycteris daviesi		1			
Lophostoma silvicolum	1	3	3		
Micronycteris brachyotis	1				
Micronycteris minuta		1			
Mimon crenulatum			3		
Phyloderma stenops		1	1		
Phyllostomus elongatus	1	15	9	21.15	<0.001*
Phyllostomus hastatus	1	3	11	11.06	0.004*
Tonatia saurophylla			1		
Tonatia sp.	1				
Trachops cirrhosus		8	2		
Glossophaginae					
Glosophaga soricina			1		
Lonchophylla thomasi	1	2	7		
Carolliinae					
Carollia brevicauda	3	18	29	24.6	<0.001*
Carollia castanea		2	7		
Carollia perspicillata	7	40	26	25.76	<0.001*
Carollia	1	1	2		
Rhinophylla pumilio		24	10	33.51	<0.001*
Stenodermatinae					
Artibeus anderseni	6	2	1		
Artibeus cinereus	1				
Artibeus concolor	2				
Artibeus glaucus	9		3		
Artibeus hartii		1			
Artibeus lituratus	208	26	21	251.95	<0.001*
Artibeus obscurus	210	40	18	237.04	<0.001*
Artibeus planirostris	318	36	11	470.49	<0.001*
Chiroderma salvini	54			118.65	<0.001*
Chiroderma trinitatum	146	2		304	<0.001*
Chiroderma villosum	64	1		132.49	<0.001*
Mesophylla macconnelli	10	9	2	6.64	0.03
Platyrrhinus brachycephalus	72	3		139.6	<0.001*
Platyrrhinus helleri	238	4	1	480.19	<0.001*
Platyrrhinus infuscus	58	4	3	88.84	<0.001*
Platyrrhinus	8				
Sphaeronycteris toxophyllum	18			39.55	<0.001*
Sturnira lilium	29		5	118.65	<0.001*
Sturnira tildae		1	1		
Uroderma bilobatum	265	2	3	536.72	<0.001*
Uroderma magnirostris	89	8	2	142.71	<0.001*
Vampyressa pusilla	27			24.6	<0.001*
Vampyriscus bidens	89		1	186.8	<0.001*
Vampyrodes caraccioli	21			46.14	<0.001*

Cont. Table 4.4.

	Collpa	Forest	Gap	*G*	*P*
Desmodontinae					
Desmodus rotundus	2		1		
Diphylla ecaudata	1				
Thyropteridae					
Thyroptera tricolor			1		
Vespertilionidae					
Myotis megalotis			1		
Total Number of Bats	**1962**	**260**	**187**		

Table 4.5. Loading values for two principal components from the PCA of the mineral content of *Piper* and *Ficus* fruits.

Mineral	CP1	CP2
Boron	-0.116	-0.540
Calcium	0.117	-0.504
Copper	-0.319	0.353
Iron	-0.014	-0.287
Magnesium	-0.099	-0.136
Manganese	-0.309	0.117
Nitrogen	-0.476	0.060
Phosphorus	-0.334	0.072
Potassium	0.126	-0.190
Sodium	-0.291	-0.378
Sulfur	-0.415	-0.101
Zinc	-0.394	-0.131

DISCUSSION

Bats' Diets

Most stenodermatine bats in southeastern Peru are *Ficus* specialists. Furthermore, most of the stenodermatine species have a strong preference for *collpas* compared to the other principal phyllostomids (*i.e.* carolliine bats). The most common species captured at *collpas* belonged to the sub-family Stenodermatinae. Among the eight most abundant species (*Artibeus planirostris*, *Uroderma bilobatum*, *Platyrrhinus helleri*, *A. obscurus*, *A. lituratus*, *Chiroderma trinitatum*, *U. magnirostrum*, and *Vampyriscus bidens*; Table 4.4), only *A. lituratus* was more associated to a

Table 4.6. Maximum, minimum, and average concentrations of selected minerals and nitrogen in *Ficus* and *Piper* fruits collected in Los Amigos Conservation Concession, Madre de Dios, Peru. Results of the contrasts analysis of variance between *Ficus* and *Piper* species are shown by the *P* values. An asterisk indicates significant differences for alpha values corrected by the Bonferroni method.

Minerals	Units	*Ficus*					*Piper*				*P*
		Max.	Species	Min.	Species	Average	Max.	Species	Min.	Species	Average	
Calcium	%	1.808	*Ficus insipida*	0.240	*Ficus sp. 5*	0.796	0.599	*Piper sp. 4*	0.115	*Piper augustum*	0.269	< 0.01*
Magnesium	%	0.403	*Ficus americana*	0.121	*Ficus jurunesis*	0.258	0.430	*Piper sp. 3*	0.203	*Piper sp. 7*	0.256	0.30
Potassium	%	2.671	*Ficus sp. 1*	1.073	*Ficus americana*	1.876	1.955	*Piper augustum*	1.270	*Piper sp. 4*	1.637	< 0.01*
Sodium	ppm	39.391	*Ficus maxima*	5.077	*Ficus sp. 4*	17.403	46.000	*Piper sp. 4*	5.182	*Piper sp. 7*	20.628	0.03
Nitrogen	%	1.512	*Ficus maxima*	0.791	*Ficus juruensis*	1.202	2.759	*Piper sp. 5*	1.435	*Piper sp. 7*	1.757	< 0.01*

Cecropia diet than to a *Ficus* diet (Figure 4.1) and no association could be established for *U. magnirostrum* because no fecal samples for this species were collected. An explanation for the strong association of *A. lituratus* with *Cecropia* species and not with *Ficus* species as expected (Giannini and Kalko 2004) may be related to sample size. Only eleven fecal samples were collected at *collpas* from over 200 individuals captured at all site types. In general, the small percentage of samples obtained at *collpas* may be related to the protocol followed to capture bats. Because most bats captured at *collpas* were reproductively active (*i.e.* pregnant or lactating) (Bravo et al. 2008, Chapter 3), I kept them in the cotton bags for less than 30 minutes before being processed, to avoid unnecessary stress. Thus, considering that passage of seeds through the guts of bats usually takes an average of 30 minutes (Fleming 1988), bats may need more time on bags to eliminate samples (Gorchov et al. 1995, Herrera et al. 2002). Other explanations may be related to the time bats spend at *collpas. Collpa* soil in some fecal samples of stenodermatine species suggests that some bats are spending enough time to drink and to pass the muddy *collpa* water. So, fruits consumed prior to visiting *collpas* may have already been eliminated at the time bats were captured. However, although the total sample size of feces (245 samples) was small relative to the total number of bats captured (2409 individuals), the consistency in the diet composition for all bat species studied suggests that stenodermatine species in southeastern Peru are mostly *Ficus* specialists as suggested for bats in Panama, Central America (Giannini and Kalko 2004).

Contrary to most stenodermatine species that had a clear preference for *Ficus* fruits, carolliine species had a more diverse diet (Figure 4.1). All three *Carollia* species were associated to a diet composed mainly by *Piper* species (as suggested by Fleming 1988, Giannini and Kalko 2004 for Central America), but also complemented with other fruit species such as Cucurbitaceae, *Phyllodendron*, *Solanum, Vismia,* undetermined species, pulp and insects (Figure 4.1, Table 4.1-4.3). In addition, because both of the two most abundant species, *Carollia*

brevicauda and *Carollia perspicillata*, were rare at *collpas,* our results strongly suggest that *collpa* visitation may be related to a *Ficus* specialist diet.

Although *Carollia* species are usually common in open areas, they were rarely captured at *collpas.* One reason why carolliine bats are common in gaps is because some *Piper* species are relatively common in disturbed areas (*e.g.* natural gaps) and attract *Carollia* species (Dumont 2003, Thies and Kalko 2004). Furthermore, because some *Piper* species are pioneer colonizers, *Carollia* species have been suggested as indicators of habitat disturbance (Wilson et al. 1996). However, at *collpas* because of the constant trampling of small plants by larger geophagous mammals, *Piper* species are not common. So, *Carollia* species were not captured at *collpas* because they do not visit them to drink *collpa* water.

Although there was a diet preference by carolliine and stenodermatine bats for *Piper* and *Ficus* species respectively, members of both sub-families consumed *Cecropia* fruits. *Cecropia* is relatively abundant in the tropics and produces fruits constantly over time (Dumont 2003). Thus, it may be a reliable source of food for all frugivorous species (Terborgh 1986, Lovoba et al. 2003).

Fruit Minerals and Nitrogen Content

Piper and *Ficus* species consumed by frugivorous bats differed in their mineral and nitrogen concentrations (Figure 4.2). The ordination analysis and the contrasts analysis of variance showed a clear distinction in the content of some minerals in fruits of *Ficus* and *Piper* species. The content of nitrogen in *Piper* species was higher than in *Ficus* species. Similar to this result, Herbst (1986) and Fleming (1988) showed higher concentrations of nitrogen in *Piper* species than in other fruit species. Nitrogen is the main constituent of proteins, thus it is very important for animals (Morris 1991). For most frugivorous bats, the ability to meet the nitrogen and protein requirements depends on the quality of fruits. Herbst (1986) concluded that bats feeding on *Piper amalago* that contained 1.93 % of nitrogen (6 % protein) obtain adequate amounts of nitrogen for

maintenance and reproduction. But bats feeding only on *Ficus ovalis* that contained 0.57 % of nitrogen (2.1 % protein) will not even meet their maintenance requirements. Similarly, Morrison (1980) and Studier and Wilson (1991) argued that bats feeding on *F. insipida* that contains 4.8 % of protein would marginally meet the maintenance requirements, and potentially need other protein sources during reproduction. However, Wendeln et al. (2000) reported higher concentrations of protein in *F. insipida* (7.9 % in dry pulp and 8.5 % in seeds) than in all *Piper* and *Ficus* species mentioned before concluding that *F. insipida* is a good source of protein (nitrogen) for bats. In addition, Herrera et al. (2002) using stable-N isotope analysis showed that three stenodermatine bats, *Artibeus jamaicensis, Uroderma bilobatum,* and *Artibeus (Dermanura) phaeotis,* relied almost entirely on fruits to meet their nitrogen demands. Our results show that *Ficus* and *Piper* species in southeastern Peru contain higher concentrations of nitrogen than their congeners reported by Herbst (1986) and Fleming (1988). For instance, *F. maxima* contains 1.51 % of nitrogen that surpasses the concentration of 0.6 % reported for *F. ovalis*, and *Piper sp.* 5 contains 2.76 % of nitrogen, which also exceeds the concentration of 1.93 % of *P. amalago* (Herbst 1986, Fleming 1988). Thus, it seems that frugivorous bats in southeastern Peru could meet the requirements of nitrogen and protein from their fruit diets.

Ficus are calcium-rich fruits. Overall, in our study site, *Ficus* fruits contained higher calcium concentrations than *Piper* fruits (Table 4.6). For example, *F. insipida* contained 18,080 ppm, whereas *Piper sp.* 4 contained 5990 ppm. This calcium-rich pattern for *Ficus* has been observed in other sites in the tropics. O'Brien et al. (1998) reported a consistent pattern of high concentrations of calcium in figs around the tropics, suggesting them as important sources of calcium for frugivorous animals. Similarly, for Panama, Nagy and Milton (1979) and Wendeln et al. (2000) reported high calcium concentrations for 14 and two species of *Ficus* (11,600 ppm and 13,150 ppm, respectively). For southeastern Peru, Gilardi (1996) reported an average of 12,700 ppm for eight *Ficus* species. Therefore, *Ficus* specialist bats (subfamily Stenodermatinae;

Giannini and Kalko 2004) seem to obtain enough calcium from their diets. On the other hand, although *Piper* fruits contain lower calcium than *Ficus* fruits, they can provide to *Carollia* bats enough to meet their demands of calcium (2000 ppm for mice; National Research Council 1995). In addition, our results show that stenodermatine species as well as carolliine species consume *Cecropia* fruits, which contain high concentrations of calcium (13,300 ppm; Nagy and Milton 1979). Accordingly, frugivorous bats in southeastern Peru seem to meet their needs of calcium from their diets.

Fruits in the southeastern Peruvian Amazon contain low amounts of sodium relative to other areas in the tropics. I found an average concentration for *Ficus* and *Piper* fruits of 17.4 ± 11.5 and 20.63 ± 15.96 ppm respectively (Table 4.6). Similarly, Gilardi (1996) found an average sodium concentration of 28.86 ± 21.02 ppm for eight *Ficus* species collected in Madre de Dios, Peru. Because plants require low concentrations of sodium, they are expected to contain low amounts of it (Morris 1991). However, compared to other sites in the tropics (Nagy and Milton 1979, O'Brien et al. 1998, Wendeln et al. 2000), sodium seems to be more limited in fruits of southeastern Peru. For instance, *Piper* fruits collected in northeastern Peru show an average sodium concentration of 730 ± 60 ppm (Studier et al. 1995). Similarly, for Central America, Wendeln et al. (2000) reported an average sodium concentration of 1690 ppm for 14 species of *Ficus* with a maximum concentration in *Ficus glabrata* with 2800 ppm. This average concentration is about 100 times the concentration found in our study. An explanation for the differences in sodium concentrations among sites may be explained by the reduction in sodium availability in areas located further inland (Stallard and Edmond 1981). In the case of *Piper* fruits from northern Peru, historical processes such as the Mid-Miocene marine incursion through the Maracaibo Basin in northern South America (Hoorn 1993, Vonhof et al. 1998) may have affected the availability of sodium in the soils where the samples were collected. Consequently,

sodium may be more limited to vertebrates, especially folivores and frugivores, in southeastern Peru than in other regions.

Ficus and *Piper* species contain high concentrations of magnesium and potassium. The average concentration of magnesium for both genera surpassed the demands for maintenance and reproduction estimated for small mammals (500 and 600-700 ppm; National Research Council 1995). So, frugivorous bats seem to meet their magnesium demands from their diets. Although there is a significant difference in the concentrations of potassium between *Ficus* and *Piper* fruits, both genera contain enough to meet the maintenance and reproductive requirements estimated for small mammals (2000-3600 ppm; National Research Council 1995). The concentrations found in this study are similar to other localities in the tropics (Nagy and Milton 1979, Gilardi 1996, O'Brien et al. 1998, Wendeln et al. 2000).

Bats' Diets and *Collpa* Visitation

Sodium appears to be a limited nutrient for frugivorous bats in southeastern Peru. Since sodium is an essential mineral for vertebrates (Michell 1995), our results suggest that it may be limited in the diets of frugivorous bats. The minimal requirements of sodium estimated for small mammals exceed the concentrations present in the fruits analyzed. The National Research Council (1995) and Dempsey (2004) reported a minimal requirement of 500 and 600 ppm of sodium, respectively. In addition, Michell (1995) suggested that sodium requirements usually increase during reproduction. For frugivorous bats, Studier and Wilson (1991) estimated a daily requirement for an adult *Artibeus jamaicencis* at 14 mg sodium/animal/day (considering 0.6 mmol/day as minimal sodium requirement for growing rats provided by Michell 1995). Considering that *A. jamaicensis* feed on *Ficus* with concentrations ~ 500 ppm (Nagy and Milton 1979, Studier and Wilson 1991), they need to ingest approx. 30 fruits per day to meet the minimal sodium requirements. For 500 ppm of sodium, each 8-g fresh *Ficus* fruit contains about 0.8 mg of sodium (~ 80 % moisture of total fresh fruit weight). However, bats extract ~ 60 % of

fruit juice (Morrison 1980), so each fruit will provide ~ 0.5 mg of sodium. In southeastern Peru, the concentrations of sodium in *Ficus* as well as *Piper* fruits contain significantly less sodium than 500 ppm. So, to meet the minimum requirements of sodium bats would need to ingest over 100 fruits per day. Because flying to search for fruits is extremely high in energy demand (Korine et al. 2004, Speakman 2008), it is possible that bats may use less costly mechanisms to supplement their low sodium fruit diets, especially during reproduction.

In southeastern Peru, mineral-rich water accumulated in soil depressions at *collpas* attracts large numbers of stenodermatine frugivorous bats (Bravo et al. 2008, Chapter 3). Because *collpa* water contains high mineral concentrations, a potential explanation for bat *collpa* visitation is mineral supplementation (Chapter 3). Contrary to the patterns of fruit mineral content found in this study, in Chapter 3 I reported consistently high concentrations of sodium in *collpa* water across seasons and years compared to other available water sources evaluated. These complementary results strongly suggest that stenodermatine bats visit *collpas* to supplement their low-sodium fruit diets. Obtaining sodium from *collpa* water may be less costly than consuming large amounts of fruits required to obtain the minimal sodium required.

However, there is a potential cost to bats of *collpa* visitation: predatory bats (probably *Phyllostomus hastatus* or *Chrotopterus auritus*) take bats that are circling to drink (Emmons pers. comm.), and *collpas* also attract boas (*i.e.*, *Corallus hortulanus*), felid predators and owls (Emmons pers. comm.; Bravo pers. obs.). Thus, the benefits for frugivorous bats to visit *collpas*, such as the acquisition of a limited resource, may be greater than the costs it may imply.

Carolliine bat species may be also using secondary sources of sodium. Although our results show that carolliine species feed primarily on *Piper* fruits, they do not frequent *collpas* as stenodermatine species do. Carolliine bats may be using different secondary sources than *collpas* to supplement their daily sodium intake or their deficit may be less dramatic than in stenodermatines. Some insects appear to contain higher concentrations of sodium than *Piper*

fruits from southeastern Peru (Seastedt and Crossley 1981, Keeler and Studier 1992, Smedley 1996). Studier et al. (1994) reported an average concentration of 540 ppm for 181 species of lepidopterans and 1660 ppm for 43 species of coleopterans. My results of diet composition suggest that carolliine species may supplement their diets with insects (Table 4.2 and 4.3). Similarly, Fleming (1988) found that *Carollia perspicillata* supplemented its diet with insects. Even more, female reproductive bats consumed more insects than non-reproductive individuals. Also, Ascorra et al. (1996) showed that *C. castanea, C. brevicauda,* and *C. perspicillata* consumed insects as supplementary items for their *Piper* diets in southeastern Peru. Therefore, in southeastern Peru carolliine species may obtain the required amounts of sodium supplementing their fruit diet with insects. In addition, although *Piper* fruits contain low sodium concentrations, some species contain higher concentrations than *Ficus* species (*Piper sp. 4;* Table 6). So, it is possible that *Carollia* bats prefer *Piper* fruits with high sodium content decreasing their potential deficits. Moreover, *Piper* fruits contain less fiber than *Ficus,* so the ingestion of nutrients may be more efficient and less costly than *Ficus.*

In conclusion, frugivorous bats in southeastern Peru seem to have sodium-limited diets. Thus, to overcome potential shortage because of sodium-poor diets, bats may use secondary sodium sources to supplement their diets. Carolliine species, which are rare at *collpas,* may be supplementing their diets with sodium-rich insects. In contrast, stenodermatine bats may be obtaining the sodium needed to meet their minimum demands, especially during reproduction, from sodium-rich *collpa* water (Bravo et al. 2008, Chapter 3). Therefore, *collpas* may function as important secondary mineral sources for stenodermatine frugivorous species and so they should be considered important conservation targets.

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CHAPTER 5. PREFERENCE FOR *COLLPA* WATER BY FRUGIVOROUS BATS: AN EXPERIMENTAL APPROACH

INTRODUCTION

In western Amazonia large numbers of frugivorous bats congregate at *collpas* to drink water that has accumulated in soil depressions (Reid et al. 2002, Voigt et al. 2007, Bravo et al. 2008). *Collpas* are open areas in the forest often visited by geophagous animals that intentionally consume exposed soil (Brightsmith and Aramburú 2004, Montenegro 2004, Tobler 2008). These places also have been referred to as clay licks, mineral licks, or *saladeros* (Emmons and Stark 1979, Gilardi et al. 1999, Reid et al. 2002, Brightsmith 2004). I prefer calling them *collpas*, their Quechua name, to avoid implying any *a priori* specific function.

Two main hypotheses have been proposed to explain the consumption of *collpa* water by frugivorous bats: detoxification of plant secondary metabolites and supplementation of limited resources (Reid et al. 2002, Bravo et al. 2008, Voigt et al. 2008). Voigt et al. (2008) proposed detoxification as the principal explanation for bats to visit *collpas* in the lowland forests of Ecuador. Based on the evidence of high concentrations of sodium in *collpa* water ingested by frugivorous bats in southeastern Peru (Chapter 3), I suggested that *collpas* are sodium sources for frugivorous bats. In addition, I found sodium to be low in their fruit diet, further supporting the argument that *collpas* are secondary sources of sodium for those bats (Chapter 4).

Although *collpas* seem to be bat activity hotspots in some places of western Amazonia (Reid et al. 2002, Voigt et al. 2007, Bravo et al. 2008) and potential explanations have been proposed for this particular behavior (Bravo et al. 2008, Voight 2008, Chapter 3), experimental evidence to test whether bats can identify *collpa* water from other water sources is lacking. In this study, I experimentally tested the hypothesis that frugivorous bats prefer *collpa* water compared to water from a non-*collpa* source.

METHODS

The study was developed in Los Amigos Biological Station (12° 34' 09" S, 70° 06' 01" W), locally known by its Spanish acronym CICRA (Centro de Investigación y Capacitación Rio Los Amigos), in the Department of Madre de Dios, in southeastern Peru. The station is located in lowland Amazonian forests between the Madre de Dios and the Los Amigos Rivers, which is within the Moist Humid Ecological Zone (Holdridge et al. 1971). The average annual temperature from 2005 to 2007 ranged from 23.93 to 24.13°C, and average annual rainfall ranged from 2152 to 2682 mm, unevenly distributed between the wet (~ October-April) and the dry seasons (~ May-September) (Atrium 2008).

In April and August 2008, I conducted experiments to test bats' preferences for *collpa* water vs. non-*collpa* water. I selected mid-sized to large bat species that were common at *collpas* (Bravo et al. 2008). Based on those criteria, I selected three mid-to-large sized stenodermatine species: *Artibeus lituratus* (~ 70 g), *A. planirostris* (~ 60 g), and *A. obscurus* (~ 40 g). Even though reproductive females are especially common at *collpas* (Bravo et al. 2008), I did not use female reproductive individuals, to avoid causing them any unnecessary stress.

The experiments were conducted with bats kept in a flight cage. The dimensions of the flight cage were 6.1 X 4.7 x 2.4 m and it was located at about 200 m from the main CICRA station buildings. The flight cage was built in the terra firme forest beneath tree canopy shade, to avoid overheating bats during the day. To provide ventilation, the sides of the flight cage were made of greenhouse shade-net. To protect bats from rain, the top of the cage was covered with a tarp. I dug two identical rectangle holes 0.5 m apart in the center of the flight cage. In each hole, I fitted an aluminum pan of 0.5 x 0.3 x 0.05 m, to offer bats two treatments: *collpa* and non-*collpa* water.

Although large numbers of bats visit *collpas* in the study area (Bravo et al. 2008, Chapter 3), I preferred to capture bats far from *collpas* to minimize biases in the responses of bats to the experiment. Thus, I captured *Artibeus* bats using 8-10 6-m mist nets (AFO 2008) deployed along trails previously established in the floodplain and terra firme forests. I opened the nets at dusk (1745 h) and closed them at midnight. After each individual was captured, I identified, measured, weighed, and placed it in a clean cotton bag to be transferred to the flight cage. Bats that did not meet the criteria for the experiment were immediately released.

Captured bats were used only once and they were kept for no more than two nights in captivity to avoid any biases in their response to the treatments. The night bats were captured I placed them in the flight cage with *ad libitum* food (bananas) and water for acclimation. The second night I conducted an experimental trial with that particular group of bats and I released them as soon as the trial ended. I conducted a total of 10 trials with 10 different groups of 2-5 bats. Based on preliminary results, I decided to use more than one individual for each experiment. When a single individual was placed in the flight cage, it did not show a response to the treatments. However, when I placed more than one individual in the cage, a positive response was recorded (Bravo unpublished data). Owing to the low abundance of bats of the same species captured per night during the dry season of this study (August 2008), some trials included two species of bats. No aggressive interactions were recorded when more than one species was used in the trial.

To determine the water preferences of bats, I offered them two water treatments: *collpa* vs. creek/rain water. *Collpa* water was collected from a *collpa* frequently visited by bats (*Collpa* No. 1 in Bravo et al. 2008). For the non-*collpa* source I used rainwater only for one trial conducted during the rainy season (April). Creek water was used for the rest of the trials because rainwater was scarce during the dry season (August). From here on I refer to creek/rain water as

non-*collpa* water. For each trial, I randomly changed the position of the treatments. The availability of water in the *collpas* was affected by the amount of rain registered in August (28 mm for 1 – 28 August 2008; Atrium 2008). Thus, I used the same *collpa* water on two consecutive trials. Although creek water was always available, I changed it at the same time I changed the *collpa* water, to maintain standardized conditions between the two treatments.

All trials were conducted using the same protocol. At 1745 h I filled each clean pan with *collpa* or non-*collpa* water, depending on which of the two treatments had been assigned to each of the two pans. I did not provide bats food until the end of each trial; but I used banana pulp to initially attract bats to the water pans. I rubbed ripe banana pulp on the edges of both pans. Preliminary observations and experiments had suggested that this dramatically increased the likelihood that bats in the flight cage would visit the pans during the course of a night's trial. I recorded the behavior of bats with a video camera (Sony MiniDV night-shot) and an external infrared light (IR Lamp 6 by www.irlight.com). I was very careful when setting up the experiment to avoid disturbing the bats. At 1800 h I turned on the video camera and the infrared light and left the cage, leaving the video camera to record bats' behavior for an hour. At 1900 h I collected the video equipment and fed the bats with bananas. I left them for 1.5 – 2 hours, after which I captured each bat in the cage using an entomological net, gave it some water with sugar using a disposable syringe and released it. After I finished each trial, I carefully watched the video and counted the number of times bats sipped water from the *collpa* vs. non-*collpa* treatment. I determined the preference for a water source using a binomial exact test for each experiment. Then, I combined the probabilities of each independent test using Fisher's method (Sokal and Rohlf 1985).

In addition, I determined the mineral concentrations of *collpa* and non-*collpa* water used for the trials of the experiments. I collected 20 ml-samples from each water type using a sterile disposable syringe. Then, I filtered the samples using a 0.45 μm Nalgene syringe filter into a

sterile centrifuge tube. I kept samples in a dark and cold place until analysis. The Soil Testing and Plant Analysis Laboratory at Louisiana State University Agricultural Center (http://www.lsuagcenter.com) analyzed the content of calcium, magnesium, potassium, and sodium for all samples. The concentrations were provided in parts per million (ppm). I compared the mineral concentrations between *collpa* and non-*collpa* water with a Hotelling-Lawley test in a Multivariate Analysis of Variance (MANOVA) (Everitt and Horton 2006). Because water was coming from the same source, the model treated each replicate as a repeated measure. All analyses were performed in R (R Development Core Team 2007, Crawley 2007).

RESULTS

A total of 33 *Artibeus* bats were used for the experiments: 17 *Artibeus lituratus*, 12 *A. obscurus,* and 4 *A. planirostris* (Table 5.1). From those, 25 were male and 8 were female individuals. Independent results for each experiment showed that bats had a preference for *collpa* water compared to non-*collpa* water. In all 10 trials bats visited the *collpa* water treatment more often than the non-*collpa* water treatment, and in six of them bats significantly preferred *collpa* water to the non-*collpa* water (Table 5.1). In addition, the overall *P* value calculated using Fisher's method showed that bats have a significant preference for *collpa* water ($P < 0.001$).

Collpa water contained significantly higher concentrations of calcium ($P = 0.03$), potassium ($P = 0.03$), and sodium ($P < 0.01$) than non-*collpa* water. There was not a significant difference in the concentration of magnesium between treatments (Figure 5.1).

DISCUSSION

Large numbers of stenodermatine bats appear to intentionally visit *collpas* where they occur in western Amazonia. The results of this study strongly suggest that stenodermatine bats can discriminate between *collpa* water and other water sources. Therefore, the preference of stenodermatine bats for *collpa* water compared to non-*collpa* water in our experiments strongly

suggests that bats purposely seek out and visit *collpas* to drink water accumulated in soil depressions.

Table 5.1. Numbers of bats used per trial, numbers of times bats drank water from the *collpa* or non-*collpa* treatments, and *P*-values for the binomial exact test. An asterisk indicates a significant difference between treatments.

Trials	Number of bats	Species and sex	*Collpa* water	Non-*collpa* water	*P* values
1	5	*A. lituratus* (3♂, 2♀)	29	4	<0.01*
2	3	*A. lituratus* (2♂, 1♀)	8	5	0.58
3	2	*A. lituratus* (1♂), *A. planirostris* (1♀)	19	12	0.28
4	3	*A. obscurus* (2♂), *A. planirostris* (1♀)	32	12	<0.01*
5	3	*A. lituratus* (1♂), *A. obscurus* (2♂)	15	3	<0.01*
6	3	*A. lituratus* (1♂), *A. obscurus* (2♂)	25	19	0.45
7	3	*A. lituratus* (3♂)	51	20	<0.01*
8	4	*A. lituratus* (2♀), *A. obscurus* (2♂)	34	25	0.29
9	4	*A. obscurus* (3♂), *A. planirostris* (1♀)	15	4	0.01*
10	3	*A. obscurus* (3♂)	29	1	<0.01*

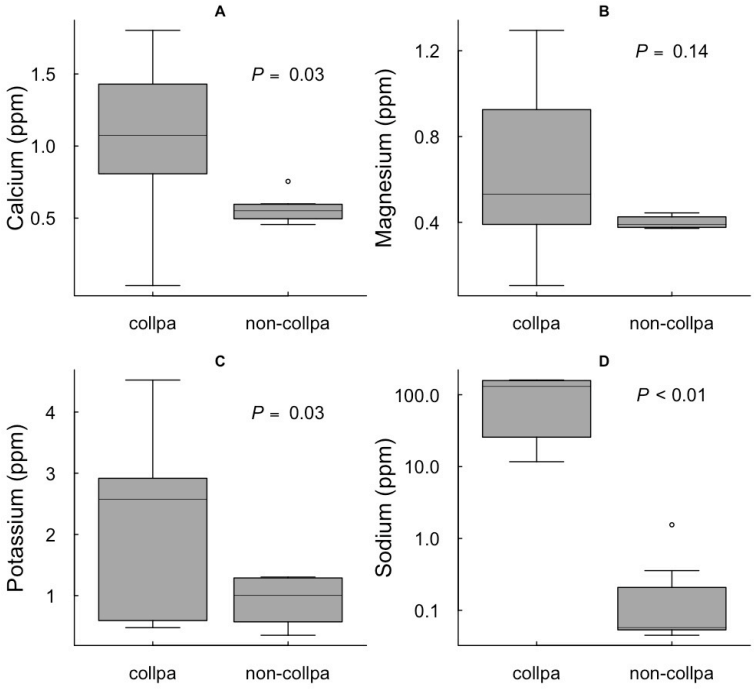

Figure 5.1. Mineral content of *collpa* and non-*collpa* water used in the choice experiments.

Contrary to insectivorous species, frugivorous bats usually obtain enough water from the fruits they consume; i.e., fruits that contain between 75-90% water (Fleming 1988, Studier and Wilson 1991); thus it is unusual for them to drink water. Therefore, it is very likely that stenodermatine bats in the Peruvian Amazon visit *collpas* seeking resources other than water.

Collpa water contains high concentrations of selected minerals. For instance, I reported a consistent pattern of high concentration of sodium in water from several *collpas* compared to creeks and oxbow lakes in southeastern Peruvian Amazonia (Chapter 3). Similarly, Izawa (1993) reported high concentrations of sodium in *collpa* water collected in Colombia. In this study, three of the four key minerals analyzed were present in significantly higher concentrations in *collpa* water compared to the non-*collpa* water, particularly sodium (average sodium concentration in *collpa* water was more than one thousand times the concentration in non-*collpa* water; Figure 5.1). Thus, it is likely, as proposed in Chapter 3, that *collpas* function as sources of limiting mineral sources for stenodermatine frugivorous bats in the Peruvian Amazon. In fact, as shown in Chapter 4 fruits consumed by stenodermatine bats in the southeastern Peruvian Amazon contained low concentrations of sodium compared to fruits collected in other tropical regions. Thus, it is likely that bats in that region face sodium constraints which may be overcome using *collpa* water as a secondary source of sodium.

Animals often seek sources of salt to supplement their diets (Denton 1982). Among many substances, salt can be accurately detected by human taste, and maybe by other mammals (Michell 1995). Because sodium is one of the most important nutrients for animals, they may search for salt in sodium-limited environments, especially species that feed primarily on plant tissues (Denton 1982, Roze 1989, Michell 1995). Contrary to animals, plants require small amounts of sodium (Morris 1991) and thus they usually contain low amounts (Nagy and Milton 1979, O'Brien et al. 1998, Wendeln et al. 2000). In this context, an appetite for salt has been suggested as one of the main drivers for the intentional consumption of sodium-rich soil, a

particular behavior defined as geophagy (Emmons and Stark 1979, Roze 1989, Holdϕ et al. 2002, Ayotte et al. 2006).

In the particular case of frugivorous bats, to determine experimentally whether bats have a preference for one or more resources present in *collpa* water, I strongly suggest the use of more choice experiments, such as those performed in this study. The results of this study are a first step towards a better understanding of the mechanism behind the behavior of bats visiting *collpas.* The experimental approach will allow us to determine whether minerals, such as sodium, as proposed in Chapter 3, or clay, as proposed by Voigt et al. (2008), present in *collpa* water are the drivers of *collpa* visitation by frugivorous bats in western Amazonia.

Contrary to other experimental studies (Giannini and Villalobos Brenes 2001, Korine and Kalko 2005, Hodgkison et al. 2007), I used groups of individuals instead of single individuals per trial because when I used single individuals in preliminary trials, they showed no response to the water treatments. In addition, contrary to food choice experiments, our experiments did not offer an obvious olfactory cue to attract bats to the treatments. I added an olfactory cue (banana pulp) in an unbiased way to both treatments, simply to increase the likelihood that bats would investigate the pans in the flight cages. *Collpa* visitation by bats may be also a social behavior. The fact that single bats did not drink water from the treatments may be explained by the potential social nature of *collpa* visitation. Although I do not precisely know whether *collpa* visitation by bats is socially facilitated, or otherwise related to social behavior, Burger and Gochfeld (2003) have described the social nature of *collpa* visitation by parrots in the Peruvian Amazon. The aggregation of large numbers of several species of frugivorous bat species, in the same way it occurs for parrots (Burger and Gochfeld 2003), suggests that inter-specific and intra-specific interactions may potentially be occurring. In addition, in the same way parrots seem to be highly vulnerable to predation while they consume the soil at *collpas* (Burger and Gochfeld 2003), there is a potential risk of predation to bats at *collpas*. Besides frugivorous bats, *collpas*

attract predatory bats (probably *Phyllostomus hastatus* or *Chrotopterus auritus*), boas (*i.e.*, *Corallus hortulanus*), felid predators and owls (Emmons pers. comm.; Bravo pers. obs.). Thus, drinking water from *collpas* with a large number of bats may decrease the probability of predation per individual. This reason may explain the behavior observed in the cages where no single individuals drank the water from any water treatment.

In conclusion, frugivorous bats appear to intentionally visit *collpas* to drink water accumulated in soil depressions made by larger geophagous mammals. Stenodermatine bats have a clear preference for *collpa* water compared to other water sources, which suggests that bats are searching for resources not provided by water sources different from *collpas.* To determine which are those resources, I strongly suggest the use of more choice experiments following the protocol described in this study.

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CHAPTER 6. CONCLUSIONS

SUMMARY

In this dissertation I studied the phenomenon of *collpa* visitation by bats in the Peruvian Amazon. More specifically, I described in detail general and seasonal patterns of use of *collpas* by frugivorous bats. Then, I addressed the hypothesis of mineral supplementation as a potential explanation for bats to visit *collpas*. And finally, I experimentally tested the preference of bats for *collpa* water compared to non-*collpa* water.

GENERAL AND SEASONAL PATTERNS OF BAT *COLLPA* VISITATION

Collpas appear to be activity hotspots for frugivorous bats in the Peruvian Amazon. Very consistently over time, large numbers of more than 20 species of frugivorous bats of the subfamily Stenodermatinae visited *collpas* to drink muddy water that had accumulated in soil depressions created by larger geophagous mammals. Contrary to stenodermatine bats, frugivorous species of the subfamily Carolliinae were among the most common species in neighboring forests and gaps but very rare at *collpas*. In addition, among stenodermatine bats at *collpas*, there was a strong female bias (> 70%) and the vast majority where reproductively active (pregnant or lactating). Therefore, *collpa* visitation by bats seems to be strongly related to frugivory and reproduction (Chapters 1 and 2).

UNDERLYING CAUSES FOR *COLLPA* VISITATION BY FRUGIVOROUS BATS

Most explanations for geophagy in the Peruvian Amazon suggest that the intentional consumption of soil is driven by the presence of high concentrations of limited key resources at *collpas* (Emmons and Stark 1979, Gilardi et al. 1999, Brightsmith and Aramburú 2004, Brightsmith et al. 2008). Because most soils in the Amazon are relatively poor in some nutrients (Stark 1970), plants may contain low concentrations of certain minerals (Nagy and Milton 1979, Gilardi 1996, Wendeln et al. 2000). As a consequence, frugivorous species may face nutritional

limitations, which may increase during periods of high nutritional demand such as reproduction. Because reproduction in bats is highly costly (Barclay 1994, Korine et al. 2004, Speakman 2008) and because *collpa* visitation is strongly associated to frugivorous bats in reproductive condition, I hypothesized that frugivorous bats visit *collpas* searching for limiting nutrients in their diets (Chapter 3).

In order to test the mineral supplementation hypothesis, I determined the mineral concentration of *collpa* water and the nutritional content of fruits consumed by bats that visit *collpas* (stenodermatines) and that generally do not (carolliines) (Chapter 3 and 4). The results showed that in fact, *collpas* are mineral-rich water sources. *Collpa* water contained higher concentrations of some key minerals for bats across seasons compared to other available water sources. Remarkably, sodium, one of the most limiting nutrients for vertebrates in the Tropics (Emmons and Stark 1979), showed a very consistent high concentration across sites and seasons. Thus, *collpas* may function as mineral sources, especially sodium, for reproductive female stenodermatine frugivorous bats in the Peruvian Amazon (Chapter 3).

To determine the nutritional content of fruits consumed by frugivorous bats that visit *collpas* and those that do not, first I determined the diet of bats captured at *collpas*, forest sites and gaps (Chapter 4). The results confirmed associations of stenodermatine and carolliine species to particular diets (Fleming 1988, Giannini and Kalko 2004). Diets of stenodermatine bats were strongly associated with *Ficus* fruits, whereas carolliine species' diets were mainly composed of *Piper* fruits and supplemented with other fruits and insects. Thus, *collpa* visitation may not be related to frugivory in general, but specifically to a *Ficus* or non-insect supplemented diet (Chapter 4).

In terms of fruit nutritional content, although *Ficus* and *Piper* species differed in their mineral and nitrogen content, both genera contained enough amounts of nitrogen and all but one mineral to reach the minimal requirements for frugivorous bats. Sodium was the only mineral

limited in the diet of stenodermatine as well as carolliine frugivorous bats. *Ficus* and *Piper* fruits contained significantly lower sodium concentrations than their congeners in other tropical regions, which suggests that sodium is very limited to frugivorous species in southeastern Peru. However, contrary to stenodermatine bats, carolliine species may be obtaining sodium from their diets. Carolliine species supplement their diets with insects, which may be sodium-rich (Studier et al. 1994). So, they may not need to visit *collpas* to supplement their diets. But stenodermatine species that are mostly *Ficus* specialists may face sodium limitations, especially during reproduction. Therefore, *collpas* may function as secondary mineral sources, especially for sodium, for reproductive frugivorous bats in the Peruvian Amazon (Chapter 4). Because of the low content of tannins in most ripe figs consumed by bats (Wendeln et al. 2000), I suggest that the hypothesis that *collpas* are sources of clay that may potentially bind secondary metabolites from bats' diets as suggested by Voigt et al. (2008) is not a likely explanation for *collpa* visitation. Thus, I maintain that mineral supplementation is a more plausible explanation for this phenomenon.

Although patterns of use of *collpas* by frugivorous bats and potential hypotheses have been proposed (Reid et al. 2000, Voigt et al. 2007, Bravo et al. 2008, Voigt et al. 2008), there was no previous experimental evidence for preferences of bats for *collpa* water compared to other water sources. In this study, I experimentally tested the preference of bats for *collpa* water (Chapter 5). The results proved experimentally that bats drink *collpa* water intentionally. Furthermore, their preferences for *collpa* water compared to other water sources indicate that *collpa* water may provide additional resources. Similar choice experiments can be used to determine which substances in *collpa* water attract bats.

Based on the evidence presented in this study of *collpas* as important mineral sources for frugivorous bats, I recommend *collpas* to be considered as important conservation targets in the Peruvian Amazon.

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APPENDIX 1. BAT SPECIES RICHNESS AND ABUNDANCES OF CHAPTER 2

Species and total captures of phyllostomid bats at *collpas* and non-*collpa* forest sites in southeastern Peru, from 94 and 616 total open net hours, respectively from 2005.

	***Collpa* sites**				**Non-*collpa* forest sites**			
Subfamily and species	**Sex**			**Total**	**Sex**			**Total**
	♀	♂	**Und.**		♀	♂	**Und.**	
Phyllostominae[a]								
Lophostoma silvicolum					1			1
Phylloderma stenops					1			1
Phyllostomus elongatus					1	1		2
Phyllostomus hastatus						1		1
Tonatia sp.			1	1				
Trachops cirrhosus					1	2		3
Glossophaginae[b]								
Lonchophylla thomasi					1			1
Carolliinae[c]								
Carollia brevicauda[d]		2		2	3	5	2	10
Carollia perspicillata[d]		3		3	4	9	2	15
Rhinophylla pumilio[e]					3	3		6
Stenodermatinae[c]								
Artibeus anderseni[f]	1		1	2				
Artibeus cinereus[f]		1		1				
Artibeus concolor[f]	1			1				
Artibeus lituratus[f]	97	19		116	4	6		10
Artibeus obscurus[f]	52	22	1	75	4	3		7
Artibeus planirostris[f]	88	20	3	111	7	2		9
Chiroderma salvini[f]	25	13	2	40				
Chiroderma trinitatum[f]	15	44	1	60				
Chiroderma villosum[f]	24	14		38				
Mesophylla macconnelli[f]	1			1	2	2		4
Platyrrhinus brachycephalus[f]	28	14		42	1	1		2
Platyrrhinus helleri[f]	116	53		169	1	3		4
Platyrrhinus infuscus[f]	11	12	1	24		1		1
Platyrrhinus spp.	2	2		4				
Sphaeronycteris toxophyllum	6			6				
Sturnira lilium[d]	10	5		15				
Uroderma bilobatum[f]	94	24		118	1			1
Uroderma magnirostrum[f]	43	9		52	1	7		8
Vampyressa pusilla[f]	9	3		12				
Vampyressa spp.	6	2		8				
Vampyriscus bidens[f]	32	18		50				
Vampyrodes caraccioli[f]	7	1	1	9				
Desmondontinae								
Desmodus rotundus[g]	1			1				
Unidentified			2	2				
Total Number of Bats	**668**	**280**	**12**	**961**	**36**	**46**	**4**	**86**

[a]Gleaning carnivores, gleaning insectivores, and omnivores that consume some fruits (Wilson 1973, Gardner 1977, Kalko and Handley 2001).
[b]Nectivores that consume some fruits and insects (Wilson 1973, Gardner 1977, Patterson et al. 1996).
[c]Frugivores (Wilson 1973, Gardner 1977, Kalko and Handley 2001, McNab 2003, Giannini and Kalko 2004).
[d]Frugivores that may specialize on Piperaceae (Wilson 1973, Gardner 1977, McNab 2003).
[e]Frugivores that may specialize on Clusiaceae (McNab 2003).
[f]Frugivores that may specialize on *Ficus* spp. (McNab 2003, Giannini and Kalko 2004).
[g]Sanguinivores that specialize on mammalian blood (Wilson 1973, Gardner 1977, McNab 2003).

APPENDIX 2. ANOVA TABLES OF CHAPTER 3

Two-way block ANOVA table for seasonal bat activity at *collpa,* forest and gap site types.

	numDF	denDF	F-value	p-value
(Intercept)	1	16	803.197	<.0001
Season	1	16	0.517	0.482
Site	2	16	316.673	<.0001
Season:Site	2	16	0.491	0.620

One-way block ANOVA with repeated measures for calcium concentrations at *collpas,* creeks and oxbow lakes in dry season of 2007.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	191.312	<.0001
Source	2	19	15.804	0.0001
Time	1	19	1.833	0.191
Source:Time	2	19	3.627	0.046

One-way block ANOVA with repeated measures for magnesium concentrations at *collpas,* creeks and oxbow lakes in dry season of 2007.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	21.883	0.0002
Source	2	19	37.213	<.0001
Time	1	19	5.033	0.037
Source:Time	2	19	2.704	0.092

One-way block ANOVA with repeated measures for potassium concentrations at *collpas,* creeks and oxbow lakes in dry season of 2007.

	numDF	denDF	F-value	p-value
(Intercept)	1	18	81.285	<.0001
Source	2	18	41.050	<.0001
Time	1	18	12.724	0.002
Source:Time	2	18	7.529	0.004

One-way block ANOVA with repeated measures for sodium concentrations at *collpas,* creeks and oxbow lakes in dry season of 2007.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	75.597	<.0001
Source	2	19	33.836	<.0001
Time	1	19	1.614	0.219
Source:Time	2	19	0.891	0.426

One-way block ANOVA with repeated measures for calcium concentrations at *collpas,* creeks and oxbow lakes of wet season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	0.024	0.877
Source	2	19	9.789	0.001
Time	1	19	0.943	0.343
Source:Time	2	19	0.130	0.878

One-way block ANOVA with repeated measures for magnessium concentrations at *collpas,* creeks and oxbow lakes of wet season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	10.437	0.004
Source	2	19	8.457	0.002
Time	1	19	0.770	0.390
Source:Time	2	19	11.751	0.001

One-way block ANOVA with repeated measures for potassium concentrations at *collpas,* creeks and oxbow lakes of wet season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	30.542	<.0001
Source	2	19	8.260	0.002
Time	1	19	0.141	0.711
Source:Time	2	19	0.527	0.598

One-way block ANOVA with repeated measures for sodium concentrations at *collpas,* creeks and oxbow lakes of wet season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	10.870	0.003
Source	2	19	98.345	<.0001
Time	1	19	0.233	0.634
Source:Time	2	19	0.249	0.781

One-way block ANOVA with repeated measures for calcium concentrations at *collpas,* creeks and oxbow lakes of dry season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	14.238	0.001
Source	2	19	6.632	0.006
Time	1	19	0.024	0.878
Source:Time	2	19	0.264	0.770

One-way block ANOVA with repeated measures for magnesium concentrations at *collpas,* creeks and oxbow lakes of dry season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	20.197	0.0002
Source	2	19	2.764	0.088
Time	1	19	0.001	0.977
Source:Time	2	19	0.04816	0.953

One-way block ANOVA with repeated measures for potassium concentrations at *collpas,* creeks and oxbow lakes of dry season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	3.335	0.083
Source	2	19	30.021	<.0001
Time	1	19	0.036	0.850
Source:Time	2	19	0.409	0.669

One-way block ANOVA with repeated measures for sodium concentrations at *collpas,* creeks and oxbow lakes of dry season 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	19	2.342	0.142
Source	2	19	250.652	<.0001
Time	1	19	0.079	0.781
Source:Time	2	19	0.091	0.912

Two-way block ANOVA with repeated measures for calcium concentrations among *collpas,* creeks and oxbow lakes of dry and wet seasons of 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	40	34.261	<.0001
Season	1	40	0.081	0.776
Source	2	40	18.863	<.0001
Time	1	40	0.699	0.408
Season:Source	2	40	1.837	0.172
Season:Time	1	40	0.410	0.525
Source:Time	2	40	0.153	0.858
Season:Source:Time	2	40	0.109	0.896

Two-way block ANOVA with repeated measures for magnesium concentrations among *collpas,* creeks and oxbow lakes of dry and wet seasons of 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	40	32.351	<.0001
Season	1	40	0.957	0.333
Source	2	40	3.220	0.050
Time	1	40	0.136	0.714
Season:Source	2	40	4.389	0.018
Season:Time	1	40	0.175	0.677
Source:Time	2	40	1.927	0.158
Season:Source:Time	2	40	2.874	0.068

Two-way block ANOVA with repeated measures for potassium concentrations among *collpas,* creeks and oxbow lakes of dry and wet seasons of 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	40	9.001	0.004
Season	1	40	1.220	0.275
Source	2	40	33.446	<.0001
Time	1	40	0.022	0.881
Season:Source	2	40	2.822	0.071
Season:Time	1	40	0.165	0.686
Source:Time	2	40	0.557	0.576
Season:Source:Time	2	40	0.390	0.679

Two-way block ANOVA with repeated measures for sodium concentrations among *collpas,* creeks and oxbow lakes of dry and wet seasons of 2008.

	numDF	denDF	F-value	p-value
(Intercept)	1	40	4.825	0.033
Season	1	40	2.932	0.094
Source	2	40	286.381	<.0001
Time	1	40	0.042	0.838
Season:Source	2	40	2.949	0.063
Season:Time	1	40	0.288	0.594
Source:Time	2	40	0.224	0.800
Season:Source:Time	2	40	0.134	0.874

APPENDIX 3. BAT SPECIES RICHNESS AND ABUNDANCES OF CHAPTER 3

Species and total captures of phyllostomid bats at *collpa*, forest and gap site types in southeastern Peru, from 167, 1280, and 558 total open net hours, respectively from 2005 to 2008.

Subfamily and species	*Collpas*				Forests				Gaps			
	Sex			Total	Sex			Total	Sex			Total
	♀	♂	Und.		♀	♂	Und.		♀	♂	Und.	
Phyllostomidae												
Phyllostominae												
Chrotopterus auritus						2		2				
Glyphonycteris daviesi						1		1				
Lophostoma silvicolum	1			1	1	2		3	2	1		3
Micronycteris brachyotis	1			1								
Micronycteris megalotis									1			1
Micronycteris minuta					1			1				
Mimon crenulatum									2	1		3
Phyloderma stenops					1			1		1		1
Phyllostomus elongatus		1		1	4	9	2	15	4	4	1	9
Phyllostomus hastatus		1		1	1	2		3	4	7		11
Tonatia saurophylla										1		1
Tonatia sp.			1	1								
Trachops cirrhosus					1	7		8	1	1		2
Glossophaginae												
Glosophaga soricina									1			1
Lonchophylla thomasi	1			1	1	1		2	3	4		7
Carolliinae												
Carollia brevicauda	1	2		3	7	9	2	18	9	19	1	29
Carollia castanea					2			2	5	2		7
Carollia perspicillata		7		7	21	16	3	40	14	11	1	26
Carollia spp.			1	1			1	1	1		1	2
Rhinophylla pumilio					14	9	1	24	3	7		10
Stenodermatinae												
Artibeus anderseni	1	5		6	1	1		2	1			1
Artibeus cinereus		1		1								
Artibeus concolor	1	1		2								
Artibeus glaucus	6	3		9					2	1		3
Artibeus hartii						1		1				
Artibeus lituratus	168	40		208	14	12		26	18	3		21
Artibeus obscurus	147	62	1	210	18	22		40	5	13		18
Artibeus planirostris	246	64	8	318	20	15	1	36	3	8		11
Chiroderma salvini	37	15	2	54								
Chiroderma trinitatum	65	80	1	146	1	1		2				
Chiroderma villosum	40	24		64			1	1				
Mesophylla macconnelli	8	2		10	4	4	1	9	1	1		2
Platyrrhinus brachycephalus	45	27		72	1	2		3				
Platyrrhinus helleri	152	85	1	238	1	3		4	1			1
Platyrrhinus infuscus	35	22	1	58	3	1		4		3		3
Platyrrhinus spp.	5		3	8								

Sphaeronycteris toxophyllum	16	2		18								
Sturnira lilium	20	9		29					3	1	1	5
Sturnira tildae						1		1		1		1
Uroderma bilobatum	194	70	1	265	2			2	1	2		3
Uroderma magnirostris	71	18		89	1	7		8	2			2
Vampyressa pusilla	21	5	1	27								
Vampyriscus bidens	56	33		89						1		1
Vampyrodes caraccioli	16	4	1	21								
Desmodontinae												
Desmodus rotundus		1	1	2						1		1
Diphylla ecaudata	1			1								
Thyropteridae												
Thyroptera tricolor										1		1
Total Number of Bats	1355	584	23	**1962**	120	128	12	**260**	87	95	5	**187**

APPENDIX 4. NUTRIENT CONTENT OF *FICUS* AND *PIPER* FRUITS ANALYZED IN CHAPTER 4

Concentrations in parts per million (ppm) and parts per hundred (%) of twelve elements for fruits of 10 species of *Ficus* and six species of *Piper* collected in Los Amigos Conservation Concession in Madre de Dios, southeastern Peru.

Species and family	Boron (ppm)	Calcium (%)	Cooper (ppm)	Iron (ppm)	Magnesium (%)	Manganese (ppm)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (ppm)	Sulfur (%)	Zinc (ppm)
Moraceae												
Ficus americana	21.717	0.850	10.225	118.184	0.395	337.711	1.232	0.128	1.006	25.561	0.113	23.266
Ficus americana	23.173	0.679	10.086	78.047	0.412	392.636	1.210	0.127	1.140	38.523	0.110	26.446
Ficus insipida	16.164	1.725	9.050	153.907	0.385	42.245	1.243	0.173	2.148	19.683	0.145	17.646
Ficus insipida	16.780	1.891	8.690	93.383	0.411	61.292	1.267	0.182	2.184	25.912	0.154	18.507
Ficus juruensis	14.662	0.860	9.784	66.680	0.115	13.673	0.764	0.137	1.335	17.365	0.064	19.161
Ficus juruensis	15.949	0.963	9.752	76.762	0.127	15.392	0.819	0.146	1.394	23.558	0.072	20.673
Ficus maxima	25.411	0.889	6.499	85.191	0.233	25.866	1.517	0.175	2.445	42.098	0.138	15.884
Ficus maxima	25.422	0.900	6.679	66.487	0.239	36.234	1.507	0.180	2.437	36.683	0.137	16.707
Ficus sp. 1	18.383	0.793	7.840	47.142	0.191	26.439	1.510	0.195	2.619	15.284	0.136	16.604
Ficus sp. 1	19.056	0.749	7.211	46.444	0.185	25.100	1.482	0.188	2.722	28.666	0.135	16.790
Ficus sp. 2	14.285	0.409	13.734	287.287	0.191	12.903	1.308	0.222	2.102	8.709	0.109	23.664
Ficus sp. 2	14.508	0.438	12.634	149.304	0.188	12.217	1.281	0.224	2.137	5.567	0.110	24.652
Ficus sp. 3	14.901	0.709	6.271	37.407	0.288	177.536	1.028	0.128	2.226	7.301	0.085	8.742
Ficus sp. 4	13.747	0.718	7.587	48.586	0.298	210.872	1.012	0.124	1.978	8.463	0.088	11.519
Ficus sp. 4	13.896	0.779	6.158	31.834	0.277	203.044	0.979	0.111	1.964	1.691	0.080	8.764
Ficus sp. 4	14.137	0.700	5.455	26.041	0.281	186.526	1.067	0.126	2.102	12.870	0.085	9.055
Ficus sp. 4	13.591	0.729	6.249	25.953	0.281	174.985	0.995	0.123	1.986	12.877	0.084	8.545
Ficus sp. 5	16.413	0.236	10.744	33.829	0.203	144.345	1.091	0.111	1.656	18.353	0.069	14.891
Ficus sp. 5	15.232	0.244	10.355	35.950	0.204	145.604	1.104	0.110	1.598	13.519	0.068	15.402
Ficus sp. 6	10.897	0.819	17.222	50.558	0.256	54.747	1.411	0.149	1.285	9.075	0.102	24.332
Ficus sp. 6	11.410	0.745	15.531	49.213	0.264	50.010	1.484	0.173	1.420	4.682	0.096	22.255
Ficus sp. 6	11.765	0.688	15.237	44.591	0.257	58.888	1.128	0.123	1.391	6.299	0.090	21.656
Piperaceae												
Piper augustum	8.901	0.120	20.147	21.091	0.262	127.558	1.719	0.193	2.190	25.531	0.138	14.683
Piper augustum	8.789	0.108	22.263	14.992	0.206	90.491	1.528	0.166	2.113	14.283	0.109	15.112
Piper augustum	8.945	0.097	22.093	13.462	0.170	127.651	1.425	0.155	2.095	14.729	0.109	15.122
Piper augustum	7.353	0.137	17.463	23.433	0.177	106.567	1.804	0.196	1.424	8.915	0.112	11.095
Piper sp. 3	9.036	0.157	17.153	42.831	0.440	644.968	1.667	0.198	1.697	12.780	0.142	11.532

Piper sp. 3	9.099	0.160	16.046	38.722	0.420	616.166	1.573	0.179	1.720	6.371	0.133	11.388
Piper sp. 4	26.208	0.630	18.962	38.214	0.202	64.571	2.187	0.212	1.440	55.289	0.141	27.495
Piper sp. 4	13.329	0.568	14.071	54.225	0.230	208.589	1.890	0.199	1.100	36.711	0.142	32.397
Piper sp. 5	21.524	0.393	19.487	38.190	0.307	918.752	2.759	0.237	1.544	39.432	0.189	51.857
Piper sp. 6	14.563	0.372	28.570	83.615	0.248	639.523	1.664	0.194	1.387	23.138	0.137	34.101
Piper sp. 7	9.370	0.251	10.233	20.307	0.210	29.435	1.368	0.278	1.489	6.769	0.155	12.871
Piper sp. 7	8.716	0.243	10.388	20.675	0.197	21.854	1.502	0.244	1.450	3.596	0.152	12.525

Replicates of one species are given by fruit samples collected from different individuals (trees for *Ficus* and shrubs for *Piper*).

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VITA

Adriana Bravo Ordoñez was born in June, 1976, in Tingo María, Peru. This city is located in the cloud forests of the Peruvian Amazon. Growing in a house surrounded by tropical rainforest awaked her curiosity and appreciation for nature. As a child, Adriana spent hours inmersed in the forest collecting plants and observing birds, fish, insects, and mammals in their natural environment. Her favorite course in primary school was natural sciences, and the best experience was a field trip to collect plants. Adriana attended the Universidad Nacional Agraria La Molina in Lima, Peru, where she obtained a bachelor degree in biology in 1998. After finishing her undergraduate studies, she took a graduate level course in environmental management. As a result, Adriana and other colleagues developed a study that evaluated the air quality in Lima city and proposed measures to improve it. With this study she was granted the title of Biologist. In 1999, she went to Cocha Cashu Biological Station in Madre de Dios, Peru, where she had the opportunity to meet extraordinary people that inspired her to continue her studies. In 2000, she took the course Ecology of Amazonian Ecosystems offered by the Organization for Tropical Studies (OTS) in northeastern Peruvian Amazon, and in 2002 an Ecology and Conservation course in Pantanal, Brazil. Adriana also worked in Tambopata with Dr. Brightsmith in the Reproductive Biology and Conservation of Macaws Project. At the same time, she worked as a naturalist guide for Rainforest Expeditions. In 2002 she joined the OTS as an assistant for a post-doctoral level course in Cocha Cashu Biological Station, where she met her adviser Dr. Harms. In 2003, Adriana joined the laboratory of Dr. Harms at Louisiana State University. After resigning the fall semester because of a tropical disease, Adriana re-joined the Harms' Lab in 2004. She defended her dissertation in April 15, 2009. Adriana wants to continue doing tropical ecology and conservation. She wants to work in Latin America.